

PROJECT TITLE :

IMMUNOLOGICAL CHARACTERIZATION OF HOST  
RESPONSE TO Schistosoma mansoni IN  
OCCUPATIONALLY EXPOSED LABORERS IN SUDAN AND  
THE INFLUENCE OF THERAPY IN IMMUNE PARAMETERS  
(5.148).

Our approach is to examine the hypothesis that irrigation canal cleaners, chronically infected and hyper exposed occupationally to S.mansoni, develop immunologic response to this parasite which are qualitatively and/or quantitatively distinct from those of other infected counterparts who are not occupationally hyper exposed.

Immunological profiles ( humoral or cellular ) which may be altered by effective treatment (praziquantel) should be related to the clinicopathological and parasitological status of individuals and their exposure history.  
The specific objectives are:

- (1) To define the immunological, parasitological and clinical status of groups of canal cleaners.
- (2) To determine the effect of chemotherapy on these parameters and observe the rate and level of reinfection.
- (3) To follow these individuals over a period of time to observe any changes in these parameters.

The following report details the study where canal cleaners and control groups were selected and investigated parasitologically, clinically and immunologically before and after treatment.

In all the groups, we were able to get a complete parasitological picture (urine and faecal), a detailed clinical picture including liver and spleen size and changes using ultra-sound. Blood samples were collected for sera, haematological and cellular studies.

OVERALL ACHIEVEMENTS:

This report details the effort exerted in achieving the objectives of the study.

- (1) The project was able to fulfill the study objectives.
- (2) Three graduate students were trained.
- (3) The scientific observations in the variety of experiments were undertaken in order to explore the

effects of both hyper- and low- exposure to S.mansoni in the Gezira.

#### Group Construction:

In order to select the groups, a survey of the area was undertaken to map the operating canals. An additional parameter was latter included to monitor water-contact and transmission of S.mansoni. This was done as part of the study in order to assess the affects of chemotherapy on reinfection and the degree of resistance to subsequent exposure.

Groups of canal-cleaners were selected from El Turabi Irrigation Centre, in the northern part of the Gezira Area. The list of workers was obtained from the Head Office in the area. A questionnaire was administered to randomly selected workers (attached). The workers and controls were divided into groups as follows:

Group 1: A total of 26 individuals were selected for this group. Nine of them resided in a small compound located near the Head Office at Turabi. The remaining seventeen lived in a small village (Kilo 7) approximately 10 kms south of the Head Office. Most of the individuals from group 1 were originally from western Sudan, immigrated to this area to take the job of canal-cleaning as a main carrier. The canal-cleaners in this group had varying years of service; however all had five years or more of continuous service. Individuals comprising this group were taken as the main subjects of occupationally hyper-exposed canal-cleaners, for more than fives years in service.

Group 2: Seventeen workers, living in a compound ( Um Jiha ) located about 30 kms north of the Head Office, made up the second group. The canal-cleaners in this group were originally Afro-Arabs from the western part of Sudan who came to Gezira Area after the drought and famine of the previous year (1985). This group of workers was included in the study to evaluate the effects of hyper-exposure to schistosome cercariae on people who had originally come from outside the endemic area. They also provided information on recently acquired acute infection.

Group 3: A group of twelve people living in the Gezira Area were also included in this study as a control group from the endemically infected area. Most of these were farmers residing in a village near Um Jiha compound or employees of the Head Office at El Turabi, working as a driver, store-keeper, etc. These will represent the normal exposure pattern in the area under study.

Group 4: Age-matched uninfected control individuals from the Khartoum area were taken to serve as control from outside the endemic area. These individuals were mainly from the working staff or University students originally from outside the Gezira Area.

Groups 1, 3 and 4 are the main groups indicated in the study. However, when we conducted the census we found a group (2) of individuals who had moved to the Gezira Area within the last year from a non-endemic area. Nonetheless, these individuals had contracted S.mansoni infection. These were incorporated into group 2. The inclusion of this new group served two purposes:

- (1) They work in the same Irrigation Office as groups 1 and 3 and ethically had to be screened and treated as part of the project services.
- (2) This group represented a recent acute infection under hyperexposed conditions and also served as a good control for comparison with the other group of canal-cleaners who had long term exposure to S.mansoni.

The study individuals were assessed parasitologically in the field for S. mansoni and S. haematobium infections. They were then transported to Khartoum in groups of 10 at a time every week for clinical and immunological studies. The clinical examination included ultrasound. Dr. Homeida examined the liver, spleen by careful palpation and then by ultrasound. The degree of Symmer's fibrosis and hepatosplenomegaly was assessed in each patient. They were then bled for haematology and immunology studies. Sera were collected and frozen for analysis of antibodies, lymphokines and monokines (immune mediators). The peripheral blood lymphocytes (PBL) were investigated for their ability to respond to mitogens and antigens. The PBL were also cultured and sensitized with mitogens and antigens for production of mediators. These were, IL-2, IFN-GAMMA, TNF and other mediators. The supernatants were collected and frozen for future analysis.

#### Parasitological Examination:

This phase was carried out in the field. Urine and stool samples were subsequently collected from the selected patients on three consecutive days. These patients were later transferred to Khartoum for clinical examination and for bleeding, and were returned to Gezira on the same day. The patients were later treated for their infection using a 40 mg/kg body-weight of praziquantel ( Biltricide, Bayer ). Consent for bleeding was only obtained on pre and 3 months post treatment periods.

At three, six, and twelve weeks post-treatment, stool samples were examined on three consecutive days for all the treated patients. A hatching test of the egg-positive stool samples was done.

#### Transmission and Water-Contact Study:

This parameter was added to the study for assessing exposure and/or resistance to reinfection. It has been suggested by Dr. Butterworth of the University of Cambridge (U.K.), based on his experience in Kenya studying the development of immunity to S. haematobium in school children, that some people are protected against reinfection while others are not. Dr. Sulaiman (co-investigator) is familiar with this type of study and was able to plan the snail surveys and water contact accordingly. The inclusion of this part did not require any further funding.

#### Results:

The parasitological data indicate that the area is a predominantly S. mansoni focus with no or very low S. haematobium infections. Only one patient had a few S. haematobium eggs. One patient had a Tenia nana infection while another had Entamoeba histolytica ( active trophozoites ). Both patients were treated after their initial examination.

#### Parasitological Picture :-

All individuals were screened by the modified Kato method for 3 consecutive days on pre- and post-treatment sampling. The canal cleaners in Group 1 had the highest mean egg count ( 678 egg/gm ), Group 2 mean ( 302 egg/gm ) and Group 3 had ( 188 egg/gm ). Six weeks after treatment all of the Group 1 individuals did not pass any eggs, and 7/27 were passing dead eggs at 3 month post-treatment. In Group 2 7/17 individuals passed viable eggs. The Group 3 did not pass any eggs at 3 month post-treatment. The treatment had a definite effect on the egg shedding and reduced the count considerably.

#### Clinical Examination:-

In total, 76 patients from the three study groups were transferred to Khartoum where they were thoroughly examined (general physical, ECG and ultrasound before and after a morning meal). A detailed history of the disease (i.e. UGI bleeding, ascites, haematuria, dysuria and previous infections) as well as previous treatment for schistosomiasis was recorded. The family history of infection with schistosomiasis was also noted. This was followed by complete physical check-up and palpation for

liver and spleen enlargements. The latter observations were confirmed by ultrasound examination.

#### Clinical Picture :-

The three groups ( 1, 2 & 3 ) varied significantly in their clinical manifestations. The chronic canal cleaners (Group 1) had advanced chronic lesions of varied grades of Symmer's fibrosis, enlarged spleens and shrunken livers. The new canal cleaners did not show any Symmer's fibrosis; but some had heptosplenomegaly. 12% of the naturally infected ( Group 3 ) had Symmer's fibrosis concurrently with shrunken livers. The first group represent a high risk group with eminent risk of bleeding due to high Symmer's fibrosis in about 100% of them. Still 100% Symmer's is high when compared to normal field infections. During the study one canal cleaner from group I died due to bleeding probably from oesophageal varices, as described by fellow individuals.

#### Immunological Studies :

This is the focus of the study and required the application of new methodology. The graduate students and the technician were not familiar with the techniques used at the outset of the study. The principal investigator, Ghalib, spent the first part training two students on the basics of sterilization, preparation of reagents, chemicals and buffers. They were then trained on tissue-culture techniques, lymphocyte purification and cultures, blastogenesis, and phenotyping of peripheral lymphocytes and monocytes.

#### Methodology :

This was mainly concerned with the humoral and cellular mechanisms. Obtaining the blood samples was the most critical factor as most Sudanese are generally apprehensive about giving blood. The purpose of the study was thoroughly explained to the study groups and their consent obtained. Approximately 30 mls of blood was collected, i.e. 10 mls for serum and 20 mls for cellular analysis. Compliance for obtaining blood samples was possible only every three months due to the reluctance of the individuals to be bled. Follow-up during the initial weeks post-treatment was not practical. Three months post-treatment was determined to be the optimal period for the blood samples collection. This permitted the investigators to assess the affects of therapy and the response to subsequent challenge.

The study groups were large and all the patients were mobile; consequently, none of them were hospitalized for further investigations. The selection of the 3 months

follow-up was decided on after discussions with Dr. Butterworth, University of Cambridge (U.K.). His advice was based on the results of his study on school children in Kenya who had been infected with schistosomiasis and later were found to have developed immunity following chemotherapy. The three months time frame was anticipated to allow major shifts in the immune status and to assess the response to subsequent cercarial challenge and reinfection rate and/or nature.

All of the study groups were clinically examined for any other illness at the time of sampling and medicines were secured for them and their immediate family members. Most of the individuals, however, came from communities that received routine minor medical care from lay 'medical assistants'. The cost of the medications dispensed to the study groups was covered by the project. The feeling of good will and confidence that we developed among our study groups facilitated blood samples collections and ensured patients co-operation.

#### Serological Studies :

The first phase was intended to produce good standard antigens from all stages of the life cycle of S. mansoni (Gezira strain). This required maintenance of an adequate aquarium, laboratory animals (mice) and trained personnel for the maintenance of the cycle and the collection of the materials for antigen preparation ( i.e. cercariae, adult worms and infected animal organs for eggs). We were successful in securing our own parasite strain antigens for the assays. The sera collected were all defibrinated, clotted, centrifuged and stored in small aliquots at -40 C or in liquid nitrogen for further analysis.

#### The studies included :

Antibody profiles in IgG, IgA, IgM and IgG subclasses 1 - 4 in the schistosomiasis patients to cercarial, adult worm antigens using antigens prepared from infected mice. Using the reference sera and antigens received from Dr. Catty, WHO-reference Lab., Birmingham, UK, and cercaria collected from infected snails. We were able to select a pool of positive and negative sera and thus standardized our test antigen, conjugate etc... However, we were able to demonstrate a strong degree of strain specificity of canal-cleaners to the Gezira strain of S. mansoni cercarial and whole worm antigens, in comparison with the antigen prepared from the Puerto Rican strain of the parasite. Our standard antigens (cercarial and whole worm) were used to screen the sera for antibody in the IgG, IgA and IgM. The positive sera were then titrated for total amounts of IgG, IgA, IgM and IgG 1-4 sub-classes antibodies to the cercarial and WWH antigen. Not all the data generated has

been analysed to date; but the attached tables comparing the response of all the study groups are shown. The canal-cleaners (Group 1) had a high IgG titre to the cercarial and worm antigens pre-treatment and did not change following chemotherapy. When some of the canal-cleaners' sera were analysed for sub-class specificity it was noticed that the chronically hyperexposed canal-cleaners mount a significant IgG4 response in comparison to the other study groups. The other study groups of the new canal-cleaners (group 2) and normally exposed contact control individuals (group 3) had lower titres initially which increased substantially following treatment. The comparative study of the classes and subclasses responses may reveal some of the mechanisms of immunity and protection against cercarial challenge.

#### Results:

The CL response were much higher against CH-antigens when compared to whole worm antigens.

The IgA and IgM responses were substantially higher in response to CH antigens when compared to WHW. The canal cleaners group 2 had very high IgM response to CH antigen which remained high following treatment in comparison to normal controls, Group 1 and 3.

The total immunoglobulin levels:

The total immunoglobulin level in the IgG class decreased following treatment in group 1, while it increased significantly in group 2 and 3. The IgM showed slight down regulation following treatment in all groups while the IgA level remained the same following treatment.

#### Cellular Studies:

- (1) complete haematology.
- (2) absolute count of eosinophils.
- (3) total count of lymphocytes isolated per ml.
- (4) phenotyping of peripheral blood cells.
- (5) blastogenesis: in-vitro stimulation of lymphocytes with mitogens and schistosome antigens.
- (6) production and assay of immune mediators IL-2 and IFN-gamma, etc.

Component no. 6 was added as it comprised an integral part of the cellular studies. However, we felt that the methodology and time used in component no. 5 would allow enough cells for studying their ability to produce immune mediators. The production and titration of these mediators will help to elucidate the immune regulation abilities of these study individuals.

We have now prepared and saved supernatants from mononuclear cell cultures from all of our study groups. Supernatants were obtained from short- and long-term cultures. Some were sensitized with mitogens (Con A and PMA), others with schistosomal antigens. Assessment of the ability to produce interleukins and gamma interferon will help to elucidate the overall ability of these individuals to regulate their immune system; together with the principal phenotypic picture of the PBL and their in-vitro response of culture to stimulants. This, in turn, will elucidate their immune response ability.

The data generated to date on the peripheral blood picture, eosinophilia, phenotyping and blastogenesis is immense and will need computation. The results have been tabulated and forwarded to our consultant and collaborator, for analysis.

The schistosomiasis patients studied were, to some degree, leukopenic and lymphopenic before treatment. Following treatment, they showed improvement. Most of them had eosinophilia. Our results indicate that hyper-exposed individuals evidenced a low response to mitogens compared to normal controls. Unexpectedly, all of the patients did not respond to schistosome antigens (CH, SEA, SWAP) before and after treatment. The antigens used in the first phase of the study were obtained from S. mansoni antigens (Puerto Rican strain) obtained from the W.H.O. reference laboratory. The absence of response may be due to the lack of antigenic specificity. The patients may be anergic to these antigens under high exposure patterns. The next phase of the cellular study will be concerned with investigating this observation. However, using Sudanese strain antigens we were unable to induce any antigen specific response.

Summary :

Long term occupational hyperexposure lead to high levels of infection and advanced irreversible pathological changes. The Symmer's fibrosis may lead to oesophageal varices with an extremely high risk of bleeding tendencies. These canal cleaners are to some degree adapted to the infection. However, their work output may be partially reduced. They remain as a continuous source of transmission. Treatment with praziquantel was very successful in clearing the infection and lead to a certain degree of protection especially in chronically infected canal cleaners. However, the advanced pathology was not reversed by chemotherapy. The immune response of all study groups, generally improved following chemotherapy. The immune response was regulated in favour of the individual. The consideration of successive chemotherapy may be beneficial and may well regulate the immune system in favour of the infected individual. The study should be extended to involve bigger groups and long

term follow up specially for parasitological, clinical and humoral parameters