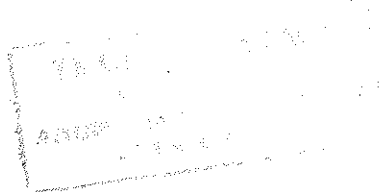


ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES



PROFILE OF ANTIBODY PRODUCTION
AGAINST *Schistosoma mansoni* IN MICE
IMMUNIZED WITH SOLUBLE ADULT WORM
ANTIGEN

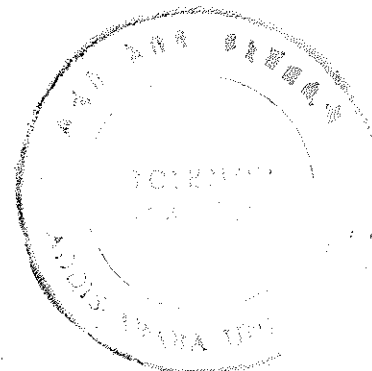


A Thesis Submitted to the School of Graduate Studies
of Addis Ababa University in Partial Fulfillment of the
Requirements for the Degree of Master of Science in
Biology
(Parasitology)

BY

GETACHEW FETAHI

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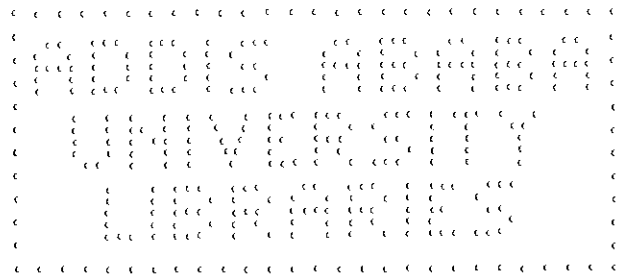
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ABSTRACT

The profile of serum antibody levels against soluble adult worm antigen (SAWA) was examined in Swiss Albino white mice over a period of 60 days of infection. A group of mice (n=15) were immunized with 0.2 mg SAWA through the oral route. Seven days after immunization, the mice were exposed to 100 cercariae of *S. mansoni*. Another group (n=15) consisted of unimmunized mice that were also exposed to 100 cercariae of *S. mansoni*. Serum samples were pooled from 4 mice within each group at 0, 7, 15, 30, 45 and 60 days post-infection and the levels of anti-SAWA antibodies determined by ELISA by using class and sub-class specific monoclonal antibodies. The results revealed significant difference in the levels of total IgG, and IgG1 antibodies in the sera of immunized mice compared to the unimmunized infected mice. In contrast, the IgM antibody responses were lower in the sera of immunized mice than in the unimmunized infected mice. Some antibody responses (IgG, IgG1 and IgM) began to rise between 15 and 30 days post-infection. Furthermore, IgG2a, IgG3 and IgE antibody responses positively correlated with the time of the onset of egg deposition. On the other hand, IgA and IgE were not detected in the immunized and the unimmunized mice. The profile of IgG secreting spleen lymphocytes from the two groups of mice showed a similar pattern of response before the onset of egg deposition (i.e. 45 days). However, the response of IgG secreting mesenteric lymphocytes was initiated earlier in the immunized mice than in the unimmunized infected mice implying that vaccination had primed the mesenteric immune apparatus. The single immunization schedule used in this study resulted in an increased level of protective antibodies (IgG and IgG1) against schistosomiasis mansoni and a decreased level of IgM antibody (which is known to be associated with blocking of protective immune response). However, the production of protective antibodies did not result in a significant reduction in the number of recovered worms. Therefore, in view of the fact that the relative quantities of protective antibodies are raised following immunization with SAWA, it will be necessary to use other parameters such as the amount and extent of granuloma formation, the viability of eggs, etc., before we conclude lack of protection by the immunization schedule used in this study.

I.INTRODUCTION

Schistosomiasis mansoni is one of the chronic and debilitating parasitic diseases of the tropics. It affects over 57 million people living in rural and agricultural areas of the world. The disease is endemic in 53 countries of Africa, Eastern Mediterranean, the Caribbean and South America. It has a negative influence on physical (growth and development of the young), social wellbeing and, seriously weakens the productive capacity of the developing countries (WHO, 1990). The spread of the disease is often associated with beneficial water development initiatives such as construction of dams and irrigation schemes. Human water use practice such as swimming, planting rice, drawing water, fishing, washing clothes, and sanitary deposition such as defecation are associated with its spread. In some countries such as Egypt, Mauritania, Senegal and Ghana, where infection with schistosomiasis mansoni has been associated with poverty, a prevalence as high as 100% has been reported (Rowe, 1984). In Ethiopia, *S.mansoni* infection has been recorded from all the regions. A prevalence of upto 95% in some endemic foci of Tigray, Wollo, Gonder and Gojjam has been reported (Birrie *et al.*, 1998)

The life cycle of *Schistosoma mansoni* involves certain species of fresh water snails as intermediate hosts, and humans and non-human primates and rodents as definitive hosts and is a highly complex one. Adult worms live in male-female pairs in hepatic portal veins, where they may survive over 20 years and each female produces about 300 embryonated eggs per day. Some of the eggs deposited into the blood stream pass through the venules and cross the intestinal wall, and reach the

lumen to be passed out with feces. Upon contact with water, the eggs hatch into the free-swimming larvae, the **miracidia**. These larvae enter the intermediate host and develop to the next larva stage known as the **cercariae**. This is the infective stage to humans. After penetration of the skin the cercariae transform into the **schistosomula**. The schistosomula through the venous routes migrate first to the lungs and then to the liver where the worms will grow to sexual maturity. Upon maturation, the male and female worms, with the female move into the gynacophoric canal of the male. The paired worms migrate down the hepatic portal vein in to the smaller mesenteric vessels.

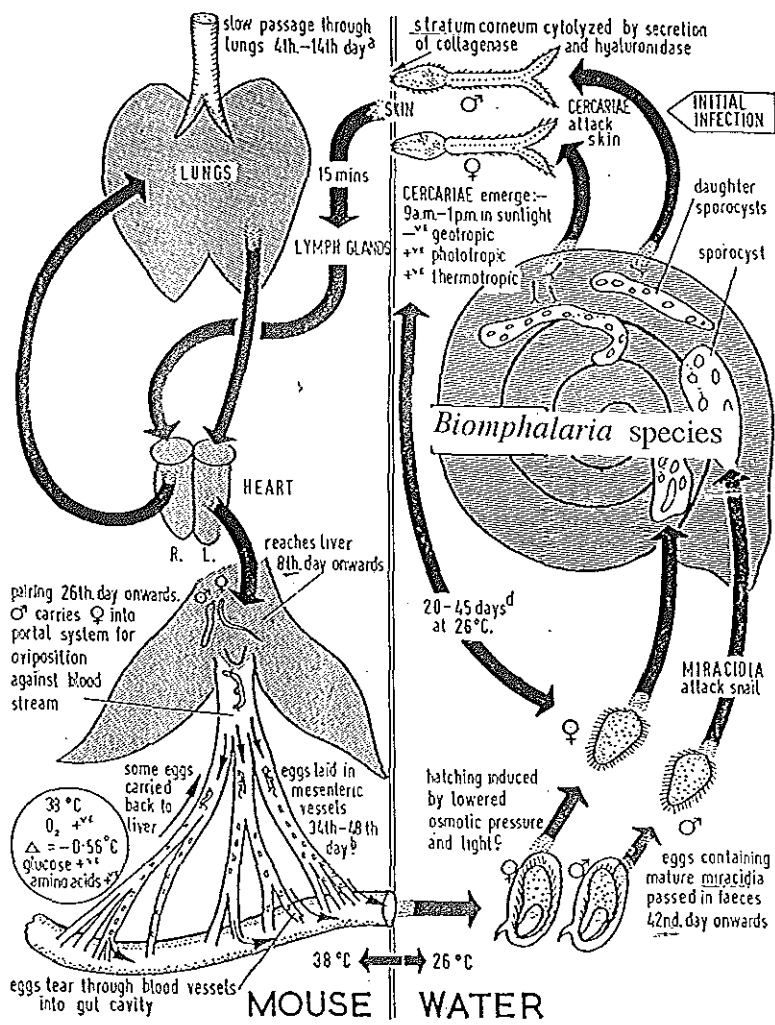


Figure 1 Life cycle of *Schistosoma mansoni* (Smyth, 1976).

Epidemiological studies in human populations show that the prevalence and intensity of schistosomiasis mansoni is an age-related event. It is very rare during the first two years of children's life, probably due to limited water contact and protection from transplacentally-transferred antibodies (Carlier *et al.*, 1980). From the age of 3 until 10 to 15 years it goes on increasing and then declines to a much lower level in the middle age group. Although variation in patterns of water contact is partly responsible for the variations in intensity and prevalence of infection between and within the age groups, recent evidences have shown that acquired immunity (Hagan *et al.* 1991) and genetic make up of the individual and the age factor are responsible for the differences in susceptibility/ resistance to reinfection (Etard *et al.*, 1995).

Disease due to schistosome parasite infection is associated with the different stages of the parasite. During penetration the cercariae secrete proteolytic enzymes from cephalic glands to facilitate larval invasion of the skin (Pino-Heiss *et al.*, 1986). This results in localized dermatitis, with intense itching and local edema. The lung stage schistosomula, as inferred from *in vitro* studies, synthesize and release at least 15 types of proteins during their migratory phase (Harrop and Wilson, 1993). The majority of them also die thus eliciting inflammatory reactions all around (Wilson *et al.*, 1986). Fever, pulmonary congestion, bronchitis and abdominal pain may accompany the inflammatory reactions. Different proteins, glycoproteins, carbohydrates and various metabolites from the adult worms are released into the

circulation together with digested and regurgitated food materials. Primarily the liver and intestinal wall traps proportions of eggs that do not reach the intestinal lumen when swept into the portal circulation. The eggs, with enclosed miracidia, constantly release a variety of highly immunogenic materials. The sustained exposure to these large amounts of antigens secreted or shed from the invading and developing larvae, the adult worms and eggs of the parasite give rise to a variety of humoral and cellular responses in the host which in turn lead to different pathological events (Lopes *et al.*, 1990; Khoury and Phillips, 1981). Among these, the major pathological reactions however are related to the deposition of the eggs in the host. Acute disease symptoms involve bloody diarrhoea and localized granulomatous inflammatory reaction on oviposition of female worms. The reactions are composed of epithelioid cells, plasma cells, lymphocytes and eosinophils elicited by trapped eggs. At the chronic stage the hepatic pathology and extensive fibrosis in the portal tract of the liver reduce the portal blood flow, thus causing portal hypertension and esophageal varices that eventually lead to gastrointestinal bleeding which can be fatal. In most cases, however, the liver pathology subsides and the granulomatous response modulates.

Clinical manifestation of the disease varies a great deal depending on the intensity of infection and the host resistance with a higher prevalence of morbidity to infection in children than adults (Guyat, 1995).

Immune responses manifested during the course of infection with *S. mansoni* are complex because of:

- continued presence of adult worms that are capable of inducing cross-reacting antibodies,
- dying of unknown number of worms during the migratory phase,
- the variable degree of immunity to reinfection,
- the presence of concomitant immunity that determines the extent to which an individual becomes super infected,
- variable intensity of reinfection after treatment, and
- age-dependent acquired resistance to reinfection.

As a result of such wide variations, full understanding of the immuno-epidemiology of schistosomiasis had remained elusive. There is ample evidence that antigens are shed or excreted during the course of *Schistosoma mansoni* infection, both from larval (Harrop and Wilson, 1993; Pino-Heiss *et al.*, 1986), adult (Deelder *et al.*, 1980) and egg (Nourel Din *et al.*, 1994) stages of the parasite life cycle. Exposures to these different antigens induce a variety of humoral and cellular responses both in human and experimental animals.

During murine infection, both specific and non-specific antibody responses have been reported (Bout, *et al.*, 1980). The immune system of the host produces both specific and cross-reacting (Dunne *et al.*, 1984; Lewis and Wilson, 1982) antibodies to different developmental stages of the parasite: larvae, adult and egg. The

antibody responses to these different stages of the parasite in murine schistosomiasis differed not only quantitatively but also qualitatively (Mazza *et al.*, 1990).

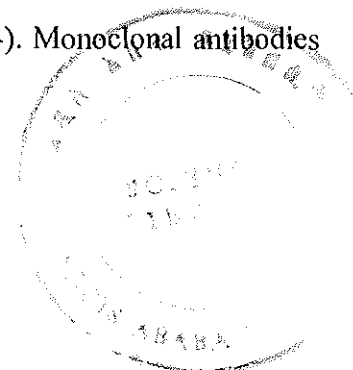
It is widely agreed that in man and experimental animals, antibodies are able to kill schistosomula *in vitro* in the presence of effector cells such as macrophages, platelets and eosinophils. The major role of antibodies in protective immunity is likely to be the induction of the cytotoxic destruction of schistosomulum. That is, antibody/complement-dependent cell-mediated cytotoxicity appears to be the major mechanism of killing of the schistosomula. On the otherhand, some antibodies have been shown to inhibit the killing activities of antibody-dependent cell-mediated cytotoxicity. Thus, resistance and susceptibility appear to be the production of different classes and subclasses of antibodies.

Most IgA antibodies are not present in the serum because their synthesis is by plasma cells in various epithelial tissues of the body that are associated with mucosa-associated lymphoid tissues (MALT). IgA is involved in protection against local infection. Mucosal immunization of mice has been reported to induce IgA response and protection (Fallon and Hagan, 1996). It has also been shown that IgA antibodies from man and the rat play a role in mediating antibody-dependent cellular cytotoxicity killing of schistosomula *in vitro* and are associated with subsequent resistance to reinfection (Dunne *et al.*, 1993; Grezel *et al.*, 1993).

In vitro studies have shown that specific IgE antibody is able to kill schistosomula in the presence of eosinophils, interferon-gamma (INF- γ) activated macrophages and platelets (Viana *et al.*, 1995, Grezel *et al.*, 1993). The killing mechanism involves antibody-dependent cell-mediated cytotoxicity. However, it has been demonstrated that although the level of parasite-specific IgE to soluble adult worm antigen (SAWA) correlates with age, there was no correlation between the level of IgE response and intensity of schistosome infection (Webster *et al.*, 1997, Vandam *et al.*, 1996; Ndhlovu *et al.*, 1996).

IgG antibodies have been shown to be directed against the protective peptide antigens found in all stages, mainly adult worm antigens. The IgG antibodies react with tegumental antigens present on larval and adult worms. The IgG antibodies have been shown to play an important role in antibody/complement-dependent cell-mediated cytotoxicity killing of schistosomula in the presence of eosinophils *in vitro* (Grezel *et al.*, 1993).

IgG1 has been suggested to be the only immunoglobulin that had specificities for the egg-derived immunogen (Bout *et al.*, 1980). However, it is demonstrated that schistosomulum membrane antigen and adult worm antigens can also elicit predominantly T-dependent IgG1 response (Mazza *et al.*, 1990). Moreover, mice exposed only to male cercariae have been shown to produce higher IgG1 antibodies to larval, worm and egg antigens (Mountford *et al.*, 1994). Monoclonal antibodies



of the IgG1 isotype raised in mice against *S. mansoni*, which recognized a carbohydrate epitope on schistosomulum surface antigens revealed a high level of complement-dependent cytotoxicity to schistosomulum *in vitro* (Yi *et al* 1986a). Similarly, IgG1 antibodies taken from humans have been shown to kill schistosomula of *S. mansoni in vitro*, in the presence of eosinophils (Dunne *et al.*, 1993, Khalife *et al.*, 1989). Passive transfer of IgG1 taken from mice polyvaccinated with irradiated cercariae of *S. mansoni* protected naïve recipients against challenge infection (Delgado and McLaren, 1990). However, lower level of IgG1 response to larval and worm antigens was reported in mice infected with attenuated cercariae than chronically infected mice (Mazza *et al.*, 1990, Mountford *et al.*, 1994). The IgG1 antibody responses do not vary with the intensity of infection (Mountford *et al.*, 1994).

Like IgG1, IgG2a antibody response is induced by schistosomulum membrane antigen and also by adult worm antigen. However, unlike the IgG1 response, the level of IgG2a response in mice infected with attenuated cercariae is higher than that in the chronically infected mice (Mazza *et al.*, 1990). In the rat, IgG2a antibody response has been shown to induce eosinophil-dependent killing of schistosomula and to confer passive protection *in vivo* (Grzych *et al.*, 1984). Moreover, IgG2a antibody responses are not affected by intensity of infection in mice (Mountford *et al.*, 1994).

Immunization of mice with adult worm antigens has been shown to produce higher levels of IgG2b antibodies against schistosome membrane antigens (Mazza *et al.*, 1990). However, elevated levels of non-specific IgG2b antibody to the parasite antigens was observed in murine schistosomiasis suggesting that the initial response of IgG2b is not specific (Khoury and Phillips 1981).

IgG2 antibody has been shown to directly block the eosinophil-dependent killing of schistosomula *in vitro*. Thus, IgG2 in humans is associated with susceptibility to schistosomiasis mansoni (Webster *et al.*, 1997).

IgG3 antibody response is mainly elicited by egg antigen (a T-dependent carbohydrate epitopes) and the response is higher after immunization of mice with soluble egg antigens (Mazza *et al.*, 1990). This response appears to block the killing activity of schistosomula.

IgM antibody has been known to be the first isotype that is synthesized after immunization or exposure with T-dependent (carbohydrate) antigens. It is mainly directed to egg and schistosomulum antigens (Omer-Ali *et al.*, 1988). It has been reported that IgM monoclonal antibodies that recognize surface carbohydrates shared between eggs, schistosomula and cercariae block antibody/complement-dependent killing of schistosomula *in vitro* (Yi *et al.*, 1986a). Furthermore, unlike other isotypes, passive transfer of IgM from polyvaccinated mice does not confer protection (Delgado and McLaren, 1990). Also, mice immunized with cholera toxin

were shown to produce high levels of specific IgM antibodies to adult worm antigens and enhanced the worm burden (Akhiani *et al.*, 1997a, 1993). In another study, cross-reactivity between *S. mansoni* adult worm antigens and cholera toxin that are specific only to the blocking antibodies of the IgM class was demonstrated (Akhiani *et al.*, 1997b). Moreover, a much lower level of IgM production has been observed in mice infected with irradiation- attenuated cercariae of *S. mansoni* than in chronically infected mice (Mazza *et al.*, 1990). In humans, IgM decreases with age and is associated with susceptibility to reinfection (Webster *et al.*, 1997; Ndhlovu *et al.*, 1996).

In contrast, another study (Viana *et al.*, 1995) demonstrated that normal individuals living in schistosome endemic areas produced a relatively higher level of IgM response against the different stages of the parasite antigens as compared to patient infection group irrespective of age.

In general, serological studies reveal disparate and sometimes contradictory results due to several factors such as the variability of experimental host, the method and technique of experimentation and the duration of the experimental period.

It has been reported that lymphocyte sub-populations in regional lymph nodes and spleen of mice exposed to primary infection with *S.mansoni* have different functions and specificities against SAWA moieties of the parasite and are directly

related to the migratory and developmental stages of the parasite life cycle (Khoury and Phillips, 1981).

Further more, a functional dichotomy in cytokine production in murine primary infection with *S. mansoni* was suggested. That is there are differences in the type and amount of antigens available to be presented from different developmental stages of the parasite, which in turn influence the classes and sub-classes of antibody responses (Mountfords *et al.*, 1994). For example, infected mice developed increased IgG1 (markers of Th2 lymphocytes) responsiveness to lung stage schistosomula, adult worms and egg antigens was evident between 5 and 7 weeks post-infection (Pearce *et al.*, 1991). In contrast, vaccinated mice developed lower levels of IgG1 and higher levels of IgG2a to larval and adult worm antigens (Mountford *et al.*, 1994).

A high level of polyclonal B-cell activation has also been observed during infection of mice with *S. mansoni*, which might be due to the presence of mitogenic products on the parasite itself (Lopes *et al.*, 1990). The proliferation of B-cells in regional lymph nodes, spleen, peripheral blood and liver granuloma has been shown only after egg deposition by the female adult worm parasites (Chennsue and Boros, 1979). *In vitro* studies of lymphocyte blastogenesis have revealed that lymphocyte proliferation before oviposition is a local rather than systemic response to schistosomula, adult worm and egg antigens following exposure to normal or irradiated *S. mansoni* cercariae (Lewis and Wilson, 1982). Lymphocyte responses

in axillary and mediastinal lymph nodes which drain the skin and lung respectively, following exposure of mice to irradiated or normal parasite showed a significant difference both in magnitude and duration which are related to variations in the pattern of parasite migration (Constant and Wilson, 1992; Constant *et al.*, 1990). The feature of antibody secreting cell (B-cells) responses in regional lymphoid organs and spleen against schistosomula, adult worm and egg antigens differ with regard to kinetics, magnitude and isotype distribution (Czerkinsky *et al.*, 1989). However the patterns of B-cell responses at local level are different from that of the serum, which is a cumulative effect of all lymphoid organs. Enzyme-linked immuno-spot (ELISPOT) assay has been found to be the best method to detect the anatomical sites of antigen specific antibody secreting cells in different tissues where immune modulation would be predicted to occur (Czerkinsky *et al.*, 1989).

Protective immunity to reinfection with *S. mansoni* can be induced in experimental animals by chronic infection, immunization with optimally irradiated cercariae, active immunization with parasite immunogens or by passive immunization with chronically infected host serum. Resistance to reinfection with *S. mansoni* has also been found after chronic infection in humans. According to Wilson (1990) the resistance induced after primary chronic infection is not immunologically linked. On the otherhand, the consequence of egg deposition and hepatic granuloma formation affect the hepatic portal circulation and this limits super-infection by preventing challenge schistosomula after their normal migration to the liver. However, the resistance to challenge infection appears to be mediated by

immunologic reaction against schistosomula that are antibody and/or cell dependent. Schistosomula stage of the parasite is thought to be the major target of protective immunity because *in vitro* studies have shown that schistosomula were found susceptible to a variety of antibody-dependent immune effector mechanisms in the presence of macrophages, eosinophils, platelets and complement (Dunne *et al.*, 1993). Adult worms evade immune response of the host principally through the acquisition of host antigens such as glycolipids, histocompatibility antigens, immunoglobulins and skin intracellular substance antigens.

Omer-Ali *et al.*, (1988) suggested that eggs are the major immunogens responsible for anti-schistosomulum surface antibody during chronic infection. However, attempts made to immunize mice with egg antigens or whole egg extracts have given negative results, and instead produced more blocking monoclonal IgM class in the sera (Yi *et al.*, 1986b). The presence of adult worms in the mesenteric veins of rhesus monkeys during primary *S.mansoni* infection or surgically implanted adult worms have been shown to induce a considerable degree of resistance and play a crucial role in concomitant immunity that protect the host against subsequent challenge infections (Smithers and Terry 1967). The induction of resistance to the challenge infection was not associated with egg production. High level of resistance was achieved by surgical implantation of 5-day-old schistosomula into the mesenteric veins of vaccinated experimental animals to bypass lung phase immunity (Delgado and McLaren, 1990). This shows that the migratory stages are not necessary to develop protective immunity.

Immunization of outbred Swiss mice through percutaneous route with individual immunogen of SAWA did not induce protective immunity, whereas that with a cocktail of soluble adult worm antigen evoked a significant protection (47%) against *S. mansoni* infection (Ridi *et al.*, 1993). Immunization of mice subcutaneously with crude extracts of worm membrane antigens two weeks prior to infection has also been observed to enhance efficacy of praziquantel treatment (Fallon and Doenhoff, 1995). Moreover, it has been reported that mice immunized with soluble components of adult worm antigen were resistant upto 70% to challenge infection and acquired a strong anti- SAWA antibody responses (James *et al.*, 1985).

Passive transfer of serum from chronically infected mice or from poly-vaccinated mice with radiation attenuated cercariae, few hours after or before challenge, conferred some degree of resistance upon naïve recipient mice and the resistance in the latter was less than that of the donor mice (Delgado and McLaren, 1990).

Stimulation of the alimentary tract by oral immunization may induce some degree of protection to challenge infection (Nedrug *et al.*, 1987; Elson and Ealding, 1984). And yet, no data are available regarding the stimulation of alimentary tract as a target of inducing protective immunity against schistosomiasis.

By using more defined methods such as the enzyme-linked immunosorbent assay (ELISA) and the enzyme-linked immuno-spot (ELISPOT) assay, based on monoclonal antibodies and cytokines, it is necessary to evaluate the kinetics of production and the magnitude of different classes and sub-classes of immunoglobulins and the anatomical site of their isotype production and distribution in different secondary lymphoid tissues. A period of 60 days of infection of mouse with *S. mansoni* would serve as a good model system.

As female worms harbor eggs, their use in antigen preparation would contaminate the adult worm antigen. Therefore, to exclude egg antigens that may exist in the female worms, only male worms were used to prepare the soluble adult worm antigen (SAWA).

The main objective of the study was: (1) to examine the profile of antibody production in mice immunized with male adult worm antigen; (2) to test whether oral immunization of mice with male adult worm antigen has any effect on immune resistance.

III. MATERIALS AND METHODS

1. *Parasite and Host*

The life cycle of a Wonji isolate *S. mansoni* was maintained in *Biomphalaria pfeifferi* and Swiss albino white mice. Both hosts were bred in the laboratory, Department of Biology, Addis Ababa University, by using the standard laboratory animals handling procedures (Mosesson and Scher, 1968; Webbe and James, 1971).

2. *Snail Infection*

Biomphalaria pfeifferi (4 to 8 mm in diameter) were infected individually according to the method by Ward *et al*, (1988). That is, after perfusion of chronically infected mice, the liver and intestinal tissues were homogenized, eggs isolated with differential sieving, washed three times with physiological saline (0.85%) followed by cold distilled water (4°C). To induce miracidia hatching the eggs in distilled water were exposed under bright light for 2 hrs at 28°C. Five miracidia were picked with pasteur-pipette under dissecting microscope and introduced to each glass exposure vial containing 3 ml of deionized water. Individual snail was added to each vial, and kept under light. Three hours later, the snails were removed and the vials examined for the presence of free miracidia. The appearance of inflamed spots at the base of the tentacles after two days of exposure of the snails to miracidia was considered to be the mark of infection of the snails (personal communication, Negash Gemedu). Infected snails were maintained in-groups of 32 in a polythene container at a constant temperature (25°C) under continuous light for 4 weeks. The snails were then kept in the dark for one more week. After 5 weeks post- nfection, individual infected snails in each vial containing 3ml of deionized water, were induced to shed cercaria phototropically at 29-30°C for 2 hr. The Cercariae were pooled and used for infection of mice within 3 hr of emergence.

3. Antigen Preparation

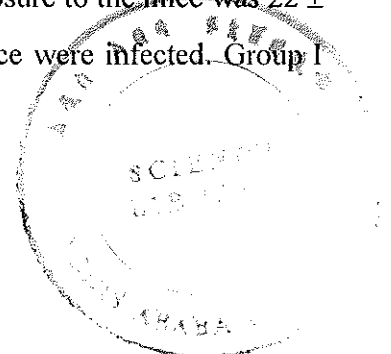
Soluble extracts of adult worm antigen were prepared as described by Mazza *et al.* (1990). Briefly, adult *S. mansoni* worms were perfused with citrate saline from 7-10 weeks-infected Swiss albino mice. The recovered worms were washed 4 times in PBS and stored at -20°C until antigen extraction. The worms were homogenized over ice for 5 minutes in PBS equal to half the volume of packed worms. The homogenates were centrifuged at 20,000xg for 2hr at about 10°C. The clear supernatant was taken as soluble adult worm antigens (SAWA). Protein content of the SAWA was determined by Folin-Ciocalteu method (Lowry *et al.* 1951) by using bovine serum albumin as a standard.

4. Immunization of Mice

A group of mice (n=15) were inoculated with 0.2 mg /mouse of *S.mansoni* male adult worm antigen in 250µl PBS (1.9 mM NaH₂PO₄, 8.1mM Na₂HPO₄; 0.138M NaCl and 0.0027M KCl, pH 7.4) orally using a 1ml syringe equipped with a 20 gauge needle with a blunted end. Age-matched group of unimmunized mice that received PBS only served as control group.

5. Infection of Mice

Prior to infection, cercarial density was determined by taking 50µl sample of the suspension drop wise onto microscope slides and staining them with iodine solution. Swiss albino white mice (5 weeks old) were exposed by tail immersion to 100 pooled cercariae of *S. mansoni* for one hr at 25-28°C. Cercariae shed from different snails were mixed to infect the mice increase the probability of both male and female worms are represented in the infection. At the end of 1hr mice were removed and their tails washed with water to examine the free cercariae left in the test tubes. The average number of free cercariae left in the test tubes following exposure to the mice was $22 \pm 1.4(M \pm SEM)$. This way, two groups of age-matched mice were infected. Group I



(n=15) was immunized with 0.2mg /mouse of male *S. mansoni* soluble adult worm antigen in PBS orally before one week of infection. Group II (n=15) was a non-immunized infected group. All mice were tested for the presence of helminth infection before use in the experiment and only those found negative were used.

6. Serum Sample Collection

Blood samples were collected from four Swiss albino mice within each group by tail bleeding after warming the mice under infrared light for 30 seconds on days 7,15,30,45, and 60 post-infection. In order to average out the variable immune responses that may occur among individual mouse immunized with SAWA, blood samples collected by tail bleeding from four mice were pooled and allowed to clot for one hour at room temperature (25°C). Serum samples from the pooled blood were collected after centrifugation (450xg) for 10 minutes. The sera were stored at -20°C until assayed.

7. Serum Antibody Levels

The levels of antibodies (all classes and subclasses) in serum samples from the three groups of mice against SAWA were determined by indirect enzyme-linked immunosorbent assay (ELISA) with class and subclass-specific anti-mouse alkaline phosphatase or peroxidase conjugated immunoglobulins by modification of existing procedures (Mazza *et al.*, 1990 and Dunne *et al.*, 1984). Optimal concentrations of 7.5ug/ml SAWA was determined by checker board titration with a reference positive serum pooled from 9 weeks infected Swiss albino mice with *S. mansoni* and a reference negative serum pooled from normal uninfected mice. Linbro/Titertek, flat bottom microtitre plates (Flow Laboratories Inc., U.S.A.) were coated with 100ul/well of 7.5ug/ml SAWA in carbonate-bicarbonate-buffer (0.015M NaCO₃, 0.035M

NaHCO₃ and 0.0031M NaN₃, pH 9.6) overnight. The plates were washed 3 times with PBS pH 7.4. To block non-specific binding, the wells were saturated with 150µl/ well of 0.05% (v/v) of Tween-20 in PBS (PBS-T) and 1%(v/v) bovine serum albumine (BSA) in PBS for one hour at 37 °C. Plates were washed three times by automated Titertek microtiter plate washer in washing buffer (PBS-T). Then after, 100µl of test sera at 1:200 dilution in blocking buffer were added to each well and incubated for two hours at 37°C. Plates washed as above and incubated for two hours at 37°C with 100 µl per well of class and sub-class specific alkaline phosphatase conjugated or horse-raddish peroxidase conjugated monoclonal antibodies at different concentration in blocking buffer. Alkaline phosphatase conjugated goat anti-mouse IgA (1: 20,000), IgG1 (1: 1500), IgG2a (1:1500), IgG2b (1:250), IgG3 (1: 250) or IgM (1: 3000), and peroxidase conjugated rabbit anti-mouse IgG (1:10,000) and rat anti-mouse IgE(1:500) were used in the assay. After three washings as above, the alkaline phosphatase and peroxidase activities were detected using 150µl/well of para-Nitrophenylene phosphate (pNPP) and Orthophenylene diamine (OPD) substrates (Sigma), respectively. Hydrolysis of pNPP and OPD were carried on for one hour and 30 minutes, respectively. The pNPP reactions were stopped by adding 50µl/ well of 0.5M NaOH and the OPD reactions were stopped by adding 50µl/ well of 3M HCL. The color development was measured at wavelength 405nm for alkaline phosphatase /NPP and at 492nm for perpxidase/OPD reaction by using automatic multichanel spectrophotometer (Titertek Multiskan plus Mk II type 313, Flow laboratories, Finland). Each assay was performed in duplicate and results are presented as the mean value of specific reactivity in optical density (OD) units.

8. Preparation of Lymphoid cells

Two mice from each group were sacrificed by diethyl ether anesthetic on days 15,30,45,and 60 post-infection. Mesenteric lymph nodes and spleen were excised and the lymphoid cells separated as described previously by Constant *et al.*, (1990) and

Czerkinsky *et al.*, (1983). Pooled lymph nodes and spleens within each group were gently teased apart using curved forceps and pressed through a 250µm stainless steel mesh into cold RPMI-1640 (Sigma Chemical Company, U.S.A.). Clumps were allowed to settle for 10 minutes on ice and the supernatant with cell suspension was transferred to sterile test tubes. After centrifugation at 900rpm for 10 minutes, the pelleted cells of lymph nodes were washed once with RPMI-1640 and were resuspended in fresh medium while the pelleted spleen cells were resuspended in 0.85% ammonium chloride Tris-buffered for 5 minutes in order to lyse erythrocytes. The suspension was then underlayered with 1ml of fetal calf serum (FCS) and spun at 1500rpm for 5 minutes to remove cell debris. After two washings in cold RPMI-1640, the lymphoid cells were counted after staining with Trypan Blue exclusion dye. Viability was consistently 80% as assessed. Cell concentrations were adjusted to 5×10^6 cells/ml in fresh culture media which consisted of RPMI-1640, 1% normal heat activated fetal calf serum, 100U/ml penicillin and 100ug/ml streptomycin and kept on ice until use.

9. Detection of Antibody Secreting Cells

Specific antibody secreting cells against *S. mansoni* SAWA were determined by enzyme linked immunospot (ELISPOT) assay. The method employed was similar to that described previously by Czerkinsky, *et al.* (1988). Briefly, individual wells of nitrocellulose bottomed 96-well plates were filled with 100µl of PBS containing 50µg of SAWA. This coating concentration was found optimal in a preliminary checkerboard titration experiment. Plates were incubated overnight at 4°C filled with coating solution. The plates were washed manually three times with PBS and immersed in this buffer for 5 minutes. Wells were then emptied off wash buffer and the outer surface of the nitrocellulose membrane carefully dried with absorbent paper towel. Individual wells were saturated with 200µl 1% fetal calf serum (FCS) in PBS and incubated at 37°C for 2 hr in humidified atmosphere with 7% carbon dioxide. After

decanting the plates, 100µl of cell suspension containing 10⁶ lymphocytes/ml of mesenteric lymph nodes and spleen were added to individual wells in sets of triplicate. Plates were then incubated for 5 hr at 37°C in a CO₂ incubator. At the completion of the cell incubation period, plates were rinsed three times with PBS and three times with PBS-T and were then immersed in PBS-T for 5 minutes. The wells were emptied off wash buffer and the outer surface of the plates was blot-dried as described above. The plates were incubated overnight at 4°C with 100µl of peroxidase conjugated rabbit anti-mouse IgG, diluted 1:5000, in PBS-T containing 1% BSA. Individual wells were then rinsed four times with PBS and immersed in 0.05M Tris-buffered saline, pH 8.8, for 5 minutes prior to development. Wells were emptied off wash buffer and blot-dried as above. Individual wells were then exposed to 100ul of 3-amino-9-ethyl carbazole (AEC)/H₂O₂ solution (Sigma Chemical Company, U.S.A.) for 10 minutes for color development. The plates were rinsed 4 times with tap water. The developed plates were dried and individual wells examined for the presence of red spots. The reactions were enumerated under low magnification (X60) of stereomicroscope equipped with a vertical white light source.

10 Assay of Immune Resistance

Resistance was assayed one week after vaccination with 0.2mg of SAWA through oral route. Recovered worms from 8 vaccinated mice (V) together with age-matched 8 non-vaccinated mice (N) were infected with 100 cercaria by the tail immersion method. Portal perfusion was carried out 9 weeks post-infection and percent resistance (R) was calculated using the formula:

$$\%R = \frac{N - V}{N} \times 100$$

11. Statistical analysis

To determine the significance of differences observed between the two experimental groups of mice in their mean number of recovered worms, antibody secreting cells/ 10^6 lymphocytes between groups, and antibody responses against SAWA were analyzed statistically by Student's *t*-test with the level of significance set at $P < 0.05$. The reported data represent the mean \pm standard error of the mean ($M \pm SEM$)



III. RESULTS

1. Serum Antibody Responses to SAWA.

Serum antibody levels in immunized and unimmunized infected Swiss albino mice over a period of 60 days were measured by using 7.5µg/ ml *S.mansoni* SAWA by indirect ELISA. Optical density (OD) values were expressed as the mean absorbance of duplicate wells after subtraction of the control values. All standard errors of the mean were less than 0.07.

Figure 2 shows the *S. mansoni* SAWA specific IgG antibody responses in immunized and unimmunized infected mice against days post- infection. Serum IgG responses to SAWA were similar in kinetics in the two groups. The IgG responses started to rise sharply between 15 and 30 days after initial cercarial exposure and continued to increase over the course of experimental period in both immunized and unimmunized mice. Mice immunized with male adult worm antigen one week before infection produced a significantly higher IgG response against SAWA at days 30 and 60 post-infection compared to unimmunized infected mice ($P < 0.005$ and $P < 0.03$, respectively). However, the level of IgG on day 45 does not show significant difference between the two groups. Moreover, IgG from serum pool of *H. nana* infected mice strongly cross-reacted (70%) with SAWA of *S.mansoni* (Table 1).

Table 1. The cross-reactivity of immunoglobulins from sera of *H.nana* infected mice to 7.5 µg/ml of SAWA as determined by ELISA. Optical density (OD) values were expressed as the mean absorbance of duplicate wells after subtraction of the control values.

Immunoglobulin classes and sub-classes	Mean OD values
IgA	0.000
IgE	0.011
IgM	0.214
IgG	1.581
IgG1	0.790
IgG2a	0.370
IgG2b	0.086
IgG3	0.022

Serum IgM responses to SAWA were significantly different between the two groups. The IgM responses were found elevated between 15 and 30 days post-infection in unimmunized infected and immunized mice. The level of IgM in immunized mice is significantly lower than that of unimmunized infected mice throughout the infection period ($P < 0.02$) except on day 45 post-infection (Fig.3) The cross reactivity of IgM from sera of *H. nana* infected mice was $<10\%$ and is statistically insignificant (Table1).

Unlike the specific IgG and IgM responses to SAWA, the IgA response was very low and undetectable through out the experimental period (Fig.4).

Serum IgE responses were marginal and an increase started from 15 days post-infection both in the immunized and unimmunized infected mice (Fig. 5). The levels reached at 60 days post-infection were 2 fold ($P < 0.03$) compared to day 7 post-

infection both in the immunized and unimmunized infected mice. However, the level of IgE response did not differ between the two groups.

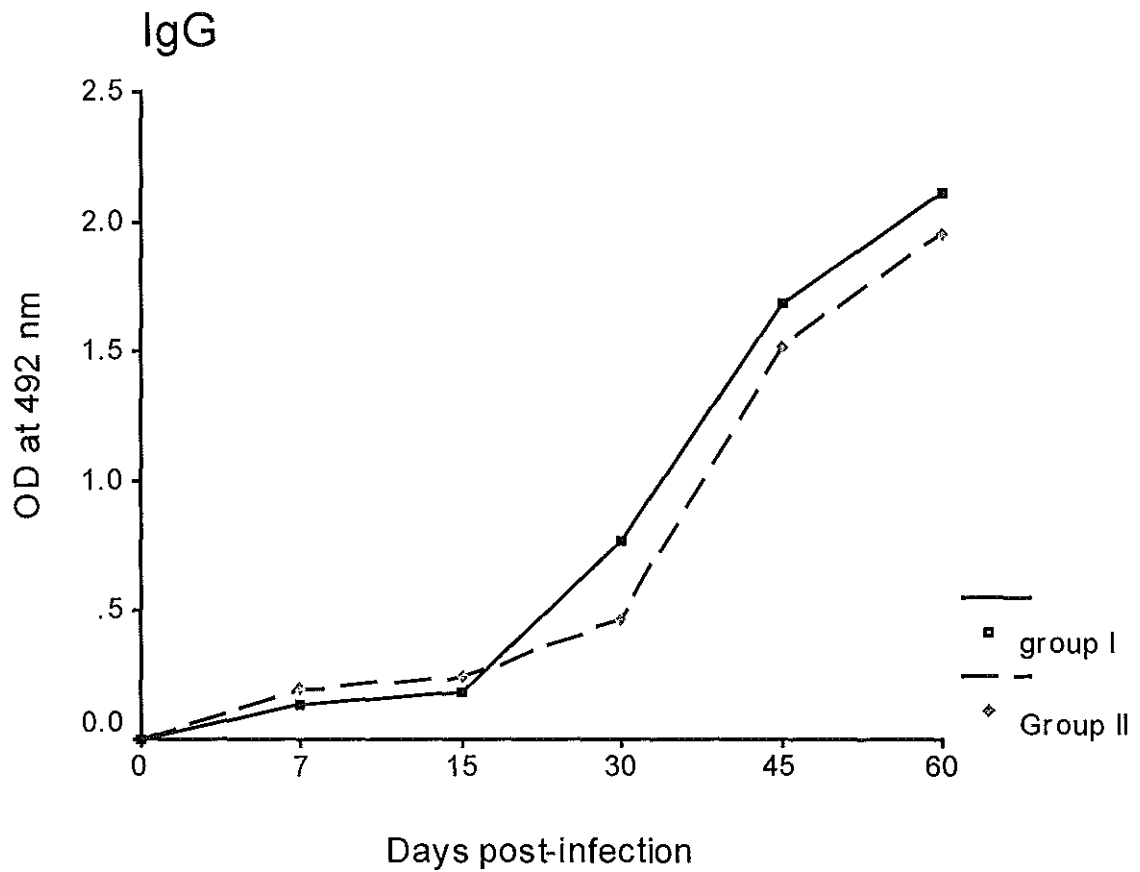


Figure. 2. The specific IgG reactivity to 7.5 μ g /ml of SAWA of *S.mansoni* as determined by ELISA. Serum pooled from 4 immunized mice (—) and 4 unimmunized mice (-----) at a dilution of 1: 200 at various days post- infection with 100 cercariae.

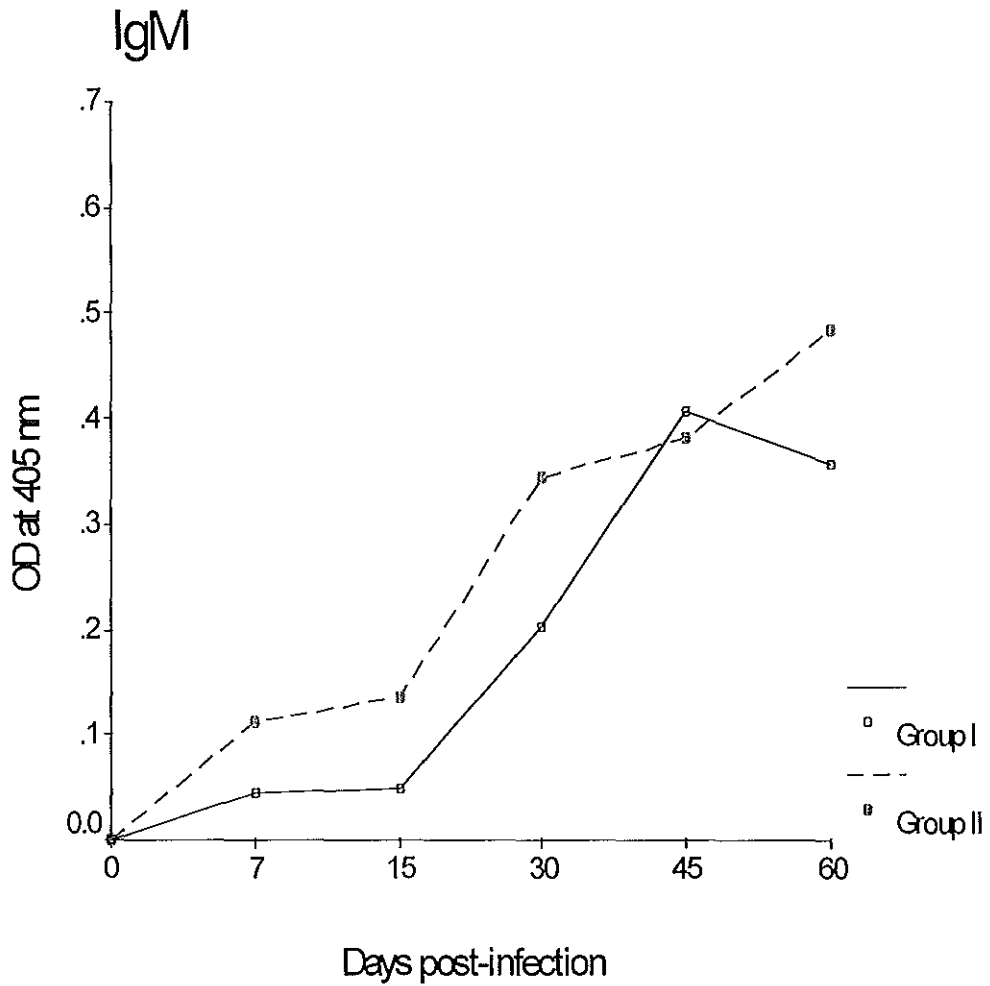


Figure. 3. The specific IgM reactivity to 7.5 μg /ml of SAWA of *S. mansoni* as determined by ELISA. Serum pooled from 4 immunized mice (—) and 4 unimmunized mice (-----) at a dilution of 1: 200 at various days post- infection with 100 cercariae.

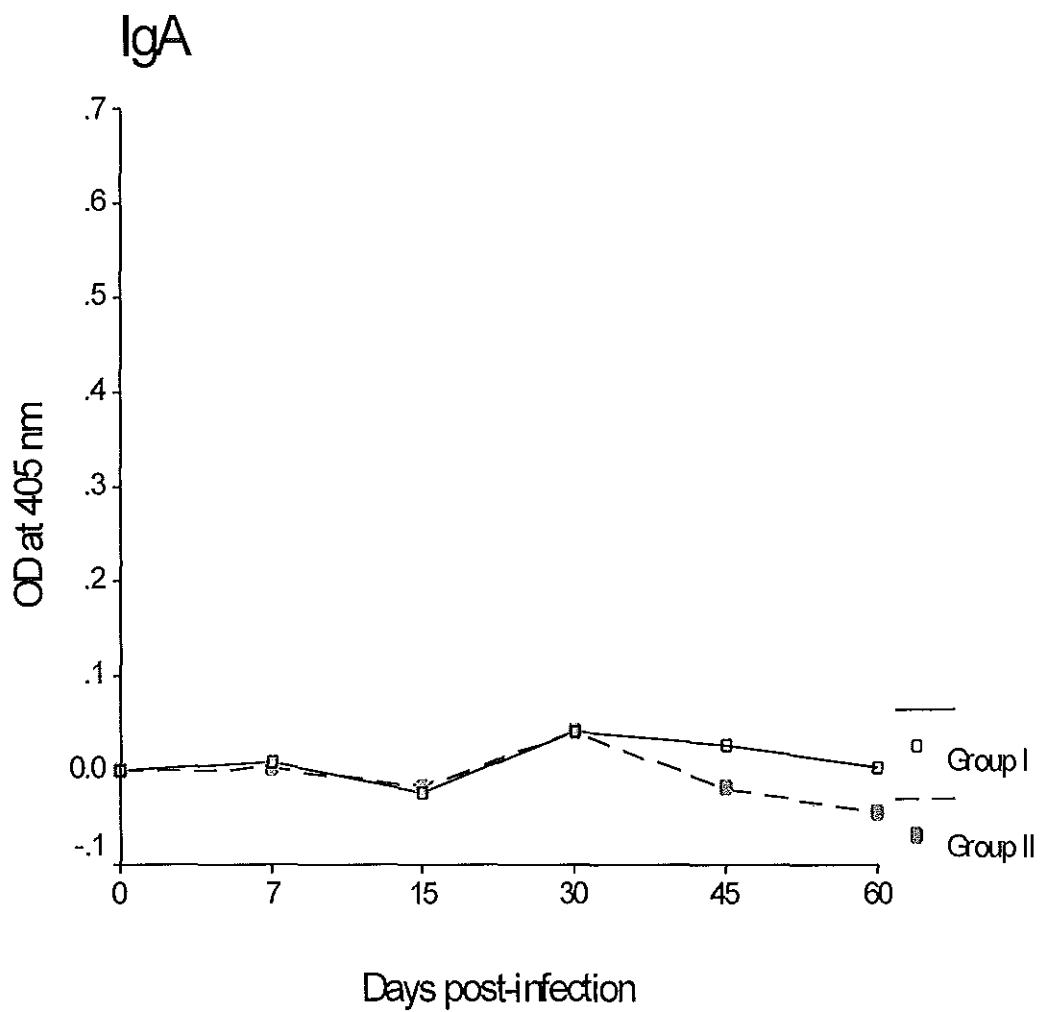


Figure 4. The specific IgA reactivity to 7.5 μ g /ml of SAWA of *S.mansoni* as determined by ELISA. Serum pooled from 4 immunized mice (—) and 4 unimmunized mice (----) at a dilution of 1: 200 at various days post-infection with 100 cercariae.

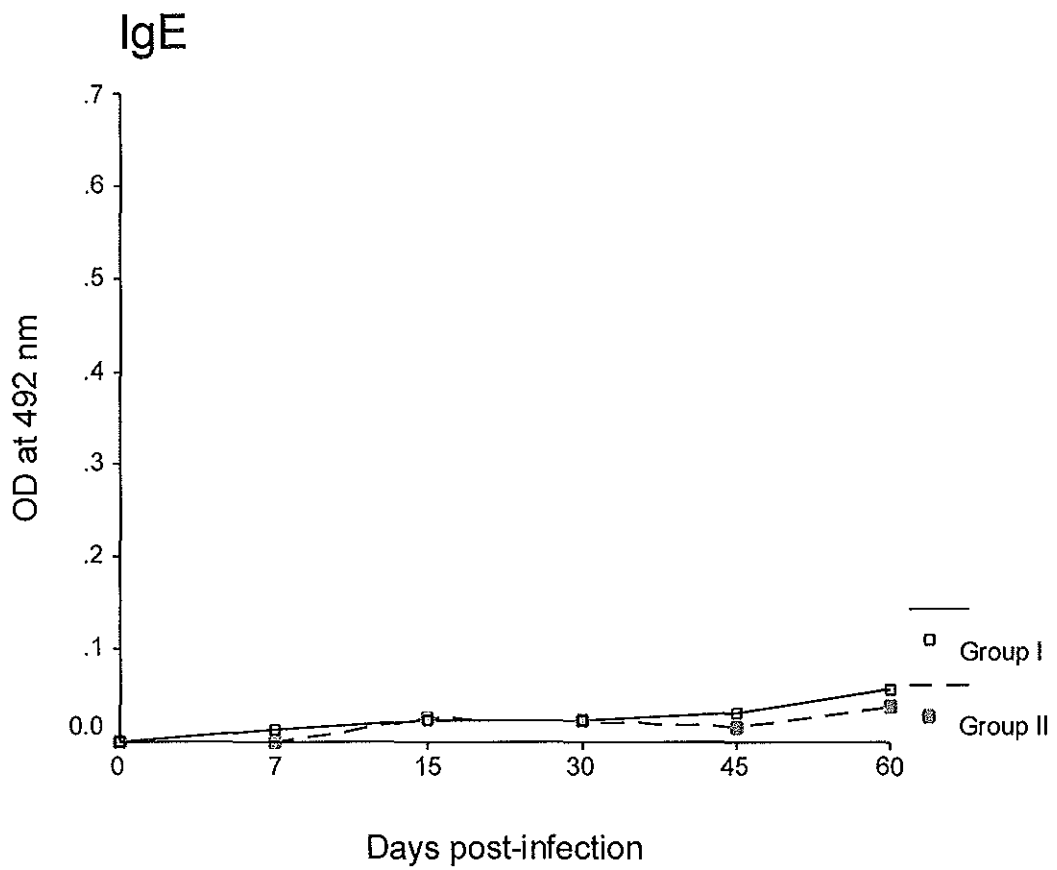


Figure 5. The specific IgE responses to 7.5 μ g /ml of SAWA of *S.mansoni* as determined by ELISA. Serum pooled from 4 immunized mice (—) and 4 unimmunized mice (-----) at a dilution of 1: 200 at various days post-infection with 100 cercariae.



The *S. mansoni*- specific antibody responses of each of the IgG sub-classes are shown in figures 6 to 9. The pronounced elevation of IgG1 responses started after 15 days post-infection in both groups of mice with peak response on day 30 post-infection. Although the level of IgG1 responses throughout the study period was relatively higher in immunized mice compared to unimmunized infected mice, a significant difference was observed at day 7 and 30 post-infection ($P < 0.04$) (Fig 6).

The IgG2a specific responses to SAWA were generally low in the sera of both groups of mice. The level started to elevate after 45 days and slowly increased ($P \leq 0.01$) to 60 days post-infection. The pattern of responses was similar between the two groups of mice (Fig. 7).

Unlike other sub-classes of IgG, the IgG2b responses started to appear in the sera of both groups of mice 7 days post-infection (Fig. 8). The pattern of responses was similar with regard to magnitude in both groups and the response patterns were irregular, showing two peaks of response on days 7 and 30 post- infection. Furthermore, IgG1 and IgG2a in the sera of *H.nana* infected mice were found to be more directed to SAWA of *S. mansoni* (Table 1).

The IgG3 responses differed very little between the two groups. In immunized-infected mice, IgG3 responses started to slightly increase between 15 and 30 days post-infection. In contrast, in unimmunized infected mice, IgG3 responses started to rise after 45-days post-infection. Thereafter, there was a slow increase in both groups of mice and the highest level was reached at day 60 (Fig. 9).

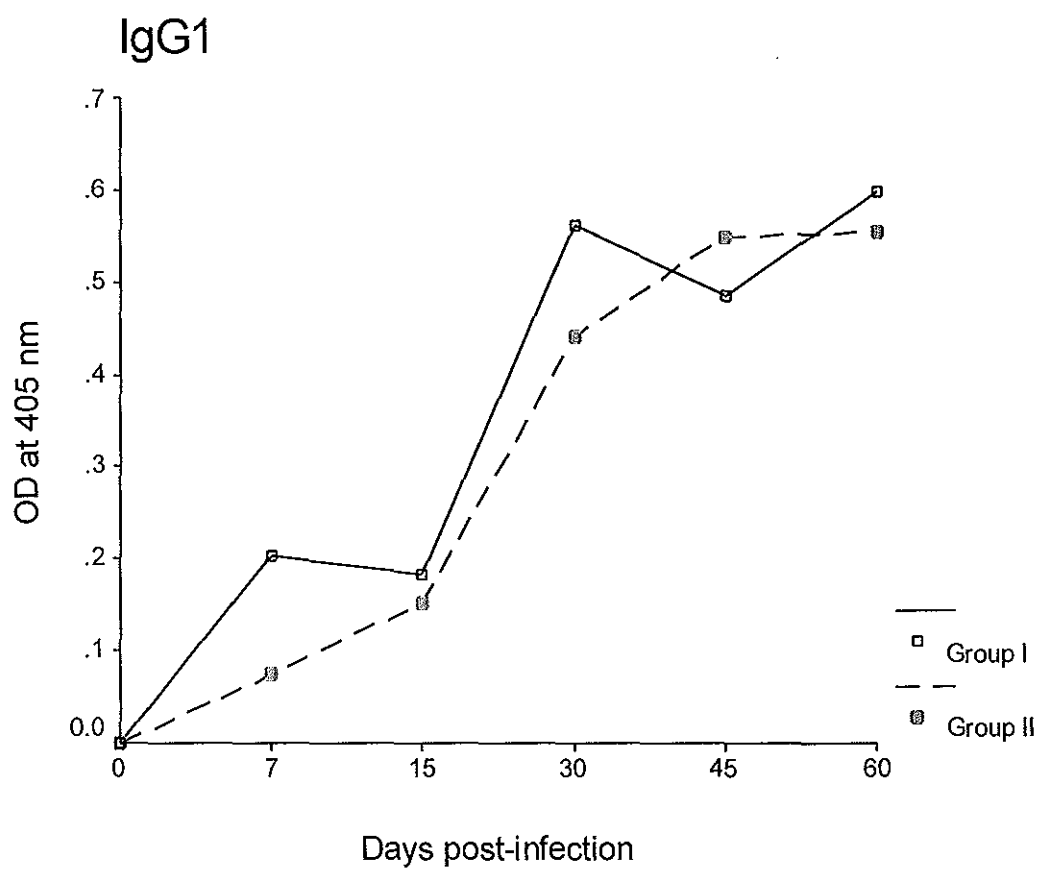


Figure 6 The specific IgG1 reactivity to 7.5 μ g /ml of SAWA of *S.mansoni* as determined by ELISA. Serum pooled from 4 immunized mice (—) and 4 unimmunized mice (-----) at a dilution of 1: 200 at various days post-infection with 100 cercariae.

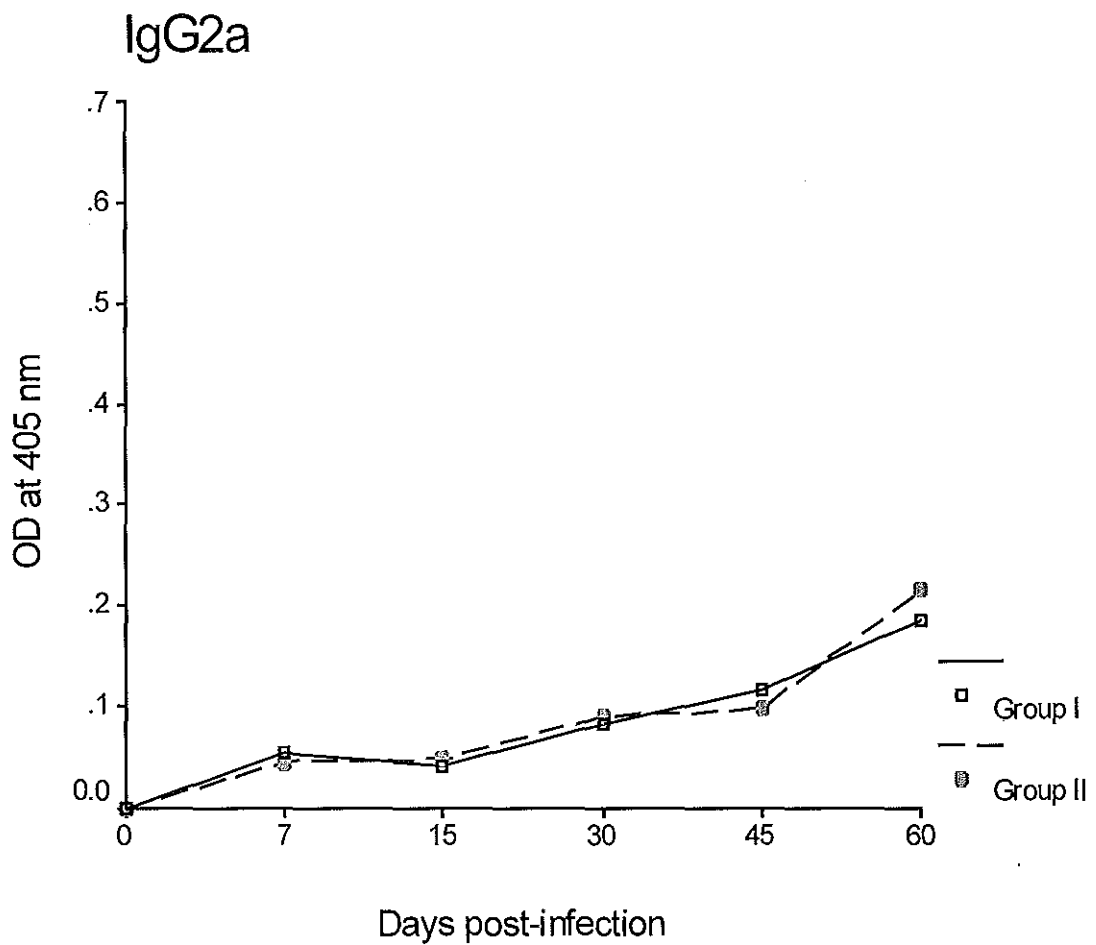


Figure 7. The specific IgG2a reactivity to 7.5 μg /ml of SAWA of *S.mansoni* as determined by ELISA. Serum pooled from 4 immunized mice (—) and 4 unimmunized mice (-----) at a dilution of 1: 200 at various days post-infection with 100 cercariae.

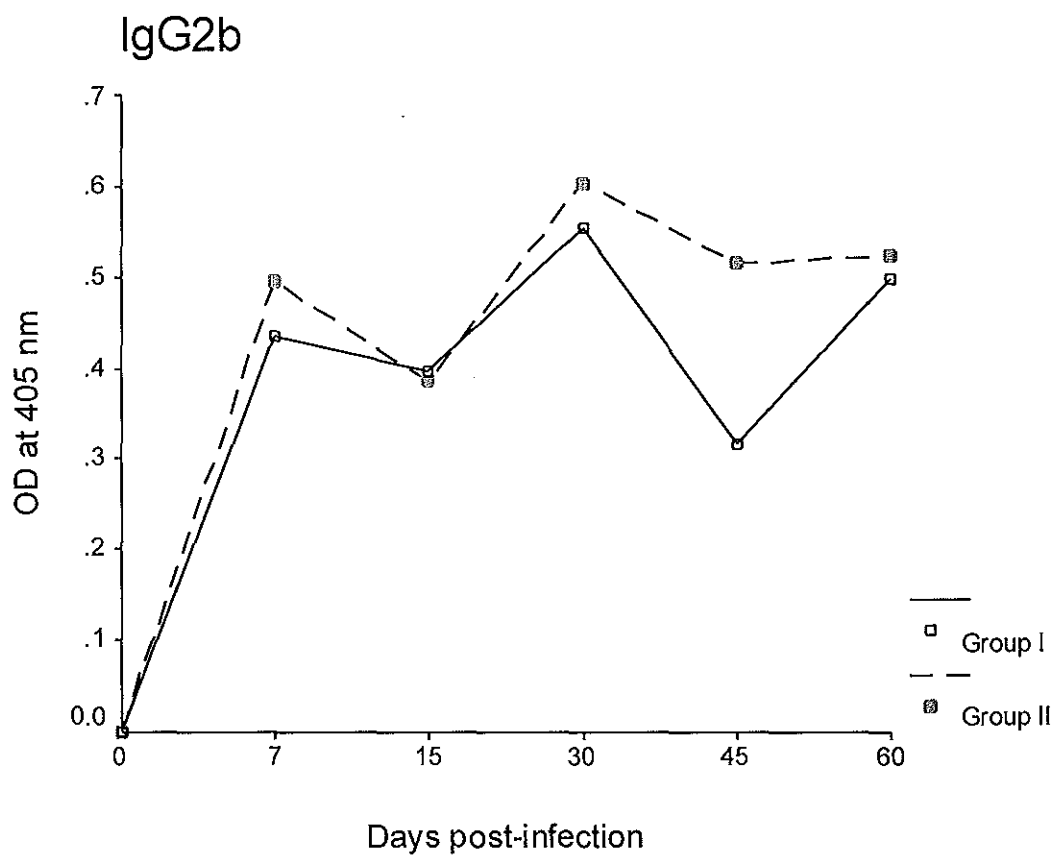


Figure 8. The specific IgG2b reactivity to 7.5 μg/ml of SAWA of *S. mansoni* as determined by ELISA. Serum pooled from 4 immunized mice (—) and 4 unimmunized mice (-----) at a dilution of 1: 200 at various days post-infection with 100 cercariae.

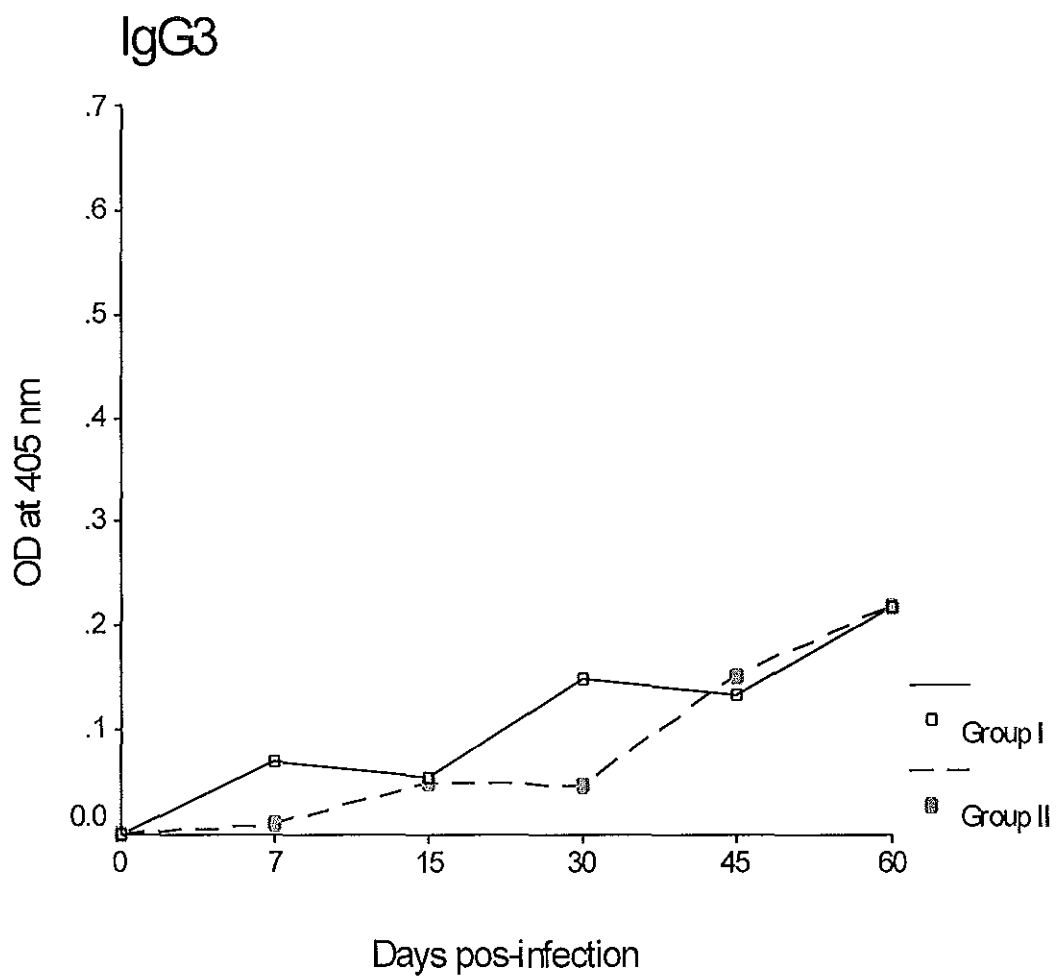


Figure 9. The specific IgG3 reactivity to 7.5 μ g/ml of SAWA of *S.mansoni* as determined by ELISA. Serum pooled from 4 immunized mice (—) and 4 unimmunized mice (-----) at a dilution of 1: 200 at various days post-infection with 100 cercariae.

2. Enumerating SAWA- reactive specific IgG antibody secreting cells

As shown in Fig.10, SAWA-reactive IgG secreting spleen cells started to rise after 15 days post-infection in both groups of mice. The number of IgG secreting cells in the spleen of unimmunized infected mice on day 60 was about 6 fold compared to day 15 post- infection. Whereas, the number of IgG secreting spleen cells in immunized mice on day 60 was about 12 fold higher than that on 15 days of infection. The kinetics of SAWA-reactive IgG secreting mesenteric lymph node cells was different between the two groups. That is, IgG secreting mesenteric lymph node cells against SAWA in immunized mice started to rise on day 30 post-infection, whereas in unimmunized mice peaked on day 60 post-infection. The response of IgG secreting cells to SAWA in mesenteric lymph nodes of immunized mice appeared earlier than that in unimmunized infected mice and the number was higher than that in unimmunized infected mice.

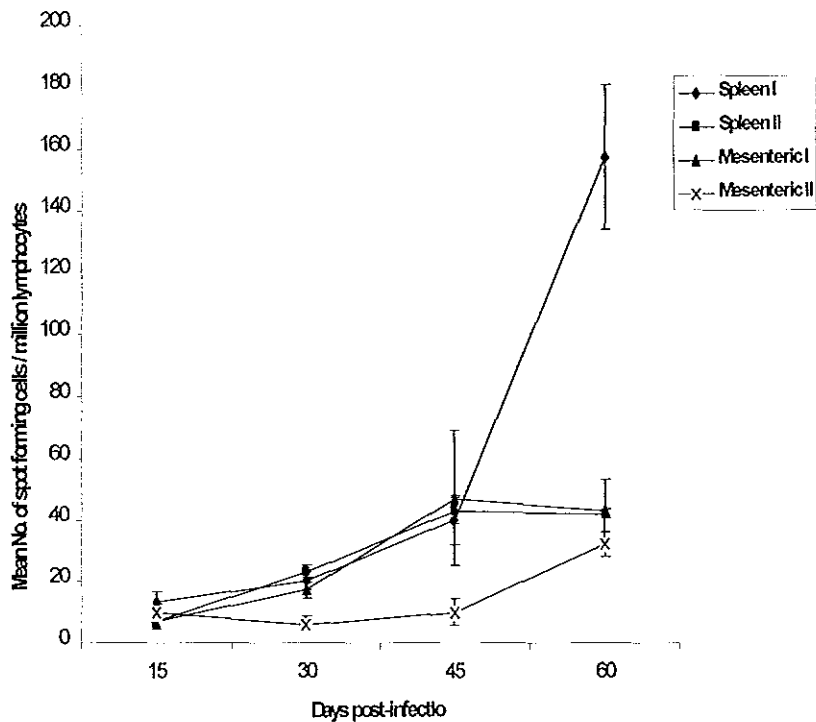


Figure 10. The number of SAWA-reactive IgG secreting cells per million of lymphocytes in the spleen and mesenteric lymph nodes of immunized (group I) and unimmunized infected mice (group II). Results are expressed as Mean (\pm SEM) of spot forming cells / 10^6 lymphocytes for triplicate wells.

3. Evaluating immune resistance

Infected mice that are immunized with 0.2mg of male SAWA /mouse orally before one week of exposure to 100 cercariae and unimmunized mice exposed to the same number of cercariae. Groups of mice (n=8) were perfused 9 weeks post- infection with citrate saline. The average number of parasites recovered (mean \pm standard deviation)

were 13.50 ± 4.1 and 10.13 ± 5.5 from immunized and non-immunized infected mice, respectively. Although more adult worms, on the average, were recovered from the unimmunized mice, the difference in the number of adult worms was not statistically significant ($P < 0.187$, Student's *t*-test). The results indicate that oral immunization with male soluble adult worm antigen has no effect on the resistance of *S. mansoni* in mice.

Table 2. The number of *S mansoni* adult worms recovered from mice infected with 100 cercariae/mouse from male soluble adult worm antigen immunized mice (group I) and unimmunized infected mice (group II).

mice	Group I(n=8)	Group II(n=8)
1	14	9
2	20	16
3	14	10
4	8	20
5	4	10
6	4	12
7	9	13
8	8	18
M \pm SD	10.13 ± 5.5	13.5 ± 4.1

IV. DISCUSSION

The profile of classes and sub-classes of antibodies against SAWA was determined in the sera from mice infected with *S. mansoni* as well as in sera from mice immunized with male SAWA through oral route. The study demonstrated that increased levels of SAWA-specific IgG, IgM, IgG1 and IgG2b were observed in the sera of both groups of mice. The most marked responses were initiated between 15 and 30 days post-infection in both cases. These results are consistent with those of Bout *et al.* (1980) and Mazza *et al.* (1990) who showed that antibody responses started after day 24 post-infection as determined by immunofluorescence and ELISA, respectively. The initiation of SAWA-reactive IgG, IgM, and IgG1 antibody responses against SAWA coincide with the arrival and growth of the developing worms in the liver (Khoury and Phillips, 1981). The developing larvae are expected to reach the liver after three weeks and release antigenic materials from the tegument, gut and in the form of excretory materials that are strongly immunogenic (Wakelin, 1996). These shed or excreted immunogenic materials from the developing worms have the capacity to stimulate marked SAWA-reactive antibody responses in the IgG and IgM isotypes. The level of IgG2b responses against SAWA started to increase during the initial infection period. This is because the initial responses may be non-specific (Khoury and Phillips, 1981). The levels of IgG2a and IgG3 were low before 45 days post-infection and their levels started to increase slowly after 45 days post-infection. This period is the time of egg deposition by the parasite (Lewis and Wilson, 1982). This suggests that the egg antigen elicits IgG2a and IgG3 antibody responses and common carbohydrate epitopes between the adult and the egg stage may exist.

Although the factors involved were not reported, it has been shown that infection with the cestode *Hymenolepis spp.* concomitantly protects experimental rats from infection with *S. mansoni* (Seyoum, 1982). In our study, test for cross reactivity of SAWA with *H. nana* infection has indicated very high degree of cross-reactivity to

IgG, IgG1 and IgG2a. This shows the existence of related antigens of *S. mansoni* to those of *H. nana*. Therefore, test for the presence of coinfection with other helminthes is essential to rule out such immune interaction in schistosomiasis mansoni investigations.

IgA responses to schistosomiasis (Grezel *et al.*, 1993) has been reported following subcutaneous immunization in rats. Bacterial and viral antigens given orally enhance local (intestinal) as well as serum IgA antibody responses in a mouse model system (Nedrug *et al.* 1987; Elson and Ealding, 1984). A very low level of local specific IgA antibody production has been described in the chronic phase of infection in mouse schistosomiasis (Crabtree *et al.* 1992; Bout *et al.* 1980). Although it is known that the IgA response in the serum could originate in gut associated lymphoid tissues (GALT), in the present study, SAWA-reactive serum IgA response was detected neither in the immunized nor in the unimmunized infected mice. A possible reason for this observation may be the short duration of infection in the unimmunized and the lack of optimization in the vaccine procedure followed (i.e. no booster doses and absence of adjuvants in the vaccine). Similar results were reported by Bout *et al.* (1980) and Lopes *et al.* (1990) who did not detect serum IgA antibodies in *S. mansoni* infected mice before 60 days post-infection. Moreover, defective transport of IgA by hepatobiliary tract is a major pathway of elimination of serum IgA in mouse. Low IgA response against worm antigens has also been reported in humans (Evengard *et al.*, 1994). Human IgA production has also been shown to increase with age and inhibit parasite's enzyme activity (Fallon and Hagan, 1996).

Controversial results have been reported about the role of specific serum IgE levels in resistance to reinfection in human schistosomiasis. Webster *et al.* (1997) reported that high serum IgE levels against SAWA is not related to intensity of infection with *S.mansoni*. Hagan *et al.* (1991) held that high levels of serum IgE protects against schistosome infection. Our findings supported the report of Webster *et al.* (1997) whereby both groups of mice exhibited a two-fold increase

in SAWA-reactive IgE level by 60 days post-infection. However, the level of IgE response did not differ between the two groups implying that immunization did not boost IgE mediated immune response. Moreover, although Webster *et al.* (1997) had reported the influence of concomitant infection with other parasitic helminthes, on the level of specific IgE, we found no cross-reaction of IgE from serum pools of *H.nana*-infected mice, against SAWA.

Comparison of specific antibody levels against SAWA between the two groups of mice showed the existence of significant differences in some isotypes before 45 days post-infection. After 15 days post-infection the IgG and IgG1 levels in the sera of immunized mice were significantly higher than in normal infected mice.

Like in serum IgG, IgG secreting spleen cells started to rise after 15 days post-infection both in immunized and unimmunized infected mice. The level of serum IgG and the number of IgG secreting spleen and mesenteric cells were higher in the immunized than in the unimmunized infected mice after 15 days post infection.. This shows that the difference in IgG responses in the serum of immunized and unimmunized infected mice is due to the difference in the response of IgG secreting lymphocytes. Oral immunization of antigens in mice has been shown to induce specific IgG response mainly in the spleen and mesenteric lymph nodes and result in substantial serum IgG antibody levels (Nedrug *et al.*, 1987; Elson and Ealding, 1984). This suggests that the IgG response originates in gut associated lymphoid tissues (GALT) and then disseminates to the spleen and the mesenteric lymph nodes. Therefore, it can be assumed that the higher IgG response in the present study is partly the result of GALT response to the SAWA. Compared to the level in the sera of unimmunized infected mice the amount of IgM, in the sera of immunized mice, were significantly lower before 45 days-post-infection. There are reports that specific IgG and IgG1 antibodies are involved in protection both *in vivo* and *in vitro* while IgM antibodies are involved in interfering with the protective effect of IgG antibodies (Delgado and McLaren, 1990, Akhiani *et al.*, 1997b, Yi *et al.*, 1986a,

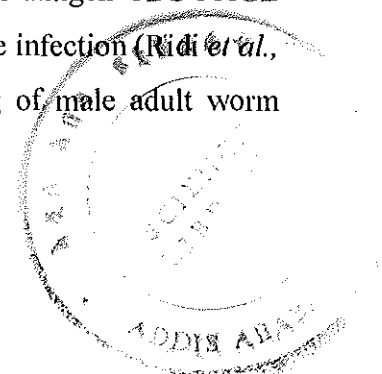
1986b). The higher level of IgG and IgG1 and the lower level of IgM in immunized mice observed in this study may be a good indication that a protective acquired immunity has been induced. Thus further, investigations to target SAWA as an oral vaccine candidate would be justifiable. Determination of the optimal dose, the use of appropriate adjuvants and the timing of booster dose before challenge infection need further investigation and understanding.

Although the level of IgG2a in immunized mice was relatively higher than in unimmunized infected mice, the difference was statistically insignificant. The IgG2a and IgG3 responses between the two groups of mice were similar both in pattern and magnitude. This could be because the two antibodies may be stimulated by thymus-independent type-2 carbohydrate epitopes shared by the egg and SAWA antigens (Mazza *et al.*, 1990). This finding is in agreement with what was reported by Akhiani *et al.* (1997) that immunization of mice with SAWA does not increase the amount of IgG2a secretion.

The increasing trend seen in SAWA-reactive antibody concentration with time in both groups is in line with the pattern and magnitude of specific-antibody secreting cells against the parasite antigens in different lymphoid organs. This also follows the kinetics of parasite migration (Czerkinsky *et al.*, 1989, Lopes *et al.*, 1990, Khoury and Phillips, 1981). The IgG secreting spleen cells to SAWA started to rise after day 15 post-infection from both groups of mice. However, the number of IgG secreting cells from the spleen of immunized mice was higher than that from unimmunized infected mice. On the otherhand, the pattern of response of the specific IgG secreting mesenteric lymphocytes was quite distinct between the two groups. The IgG secreting cells from mesenteric lymph nodes of immunized mice started to rise after 15 days post-infection, whereas in unimmunized infected mice, IgG secreting cells started to rise after day 45 post-infection. Although spleen is the major center of IgG production against SAWA, the earlier response of mesenteric IgG secreting lymphocytes in immunized mice may be responsible for the increased level of serum IgG. Studies have shown

immune responses against protective schistosome antigens, in different rodent species, to be site and not stage-dependent (Delgado and McLaren, 1990, James *et al.*, 1985). Also, in mice and in humans, immunity to reinfection has been shown to coincide with the presence of adult worms (Delgado and McLaren, 1990). Moreover, it has been reported that worm elimination in both normal mice and irradiated-cerariae-immunized mice takes place in the gastrointestinal tract where unsuccessful migrating worms die after passing the alveoli and trachea (Dean and Mongold, 1992). Thus, the gastrointestinal tract may serve an important site of soluble adult worm antigen exposure to the immune apparatus in schistosomiasis *mansoni*. Antigen uptake may occur via specialized cells overlaying the Payer's patches, or trapped by surface antibodies. On the other hand, immune stimulation of mesenteric lymph nodes in the unimmunized infected mice will be expected to occur after 6 weeks when egg deposition begins (Lewis and Wilson, 1982). From the present study, it can be concluded that the relatively increased levels of serum IgG antibodies in immunized mice could be due to immune priming of the mucosal immune system against the soluble male adult worm antigen that in turn expressed itself systematically.

Attempts to immunize mice intradermally with one mg adult worm homogenate antigens have been shown to confer protection up to 70% (James *et al.* 1985). Intradermal immunizations of mice with 10µg of different stages of *S. mansoni* antigens (schistosmula, adult, and egg) with 5 boosters have been shown to induce different patterns of antibody responses (Mazza *et al.*, 1990). Mice immunized intranasally with 100µg of SAWA alone have also been shown to produce a Th1 type response in the spleen and in the lung than mice immunized with SAWA in combination with cholera toxin or cholera toxin alone (Akhiani *et al.*, 1997a, 1993). The Th1 response is suggested to be important in antigen-specific cell-mediated protective immunity (James *et al.*, 1984). Moreover, it was reported that mice immunized subcutaneously with 8µg of adult worm antigen SDS-PAGE bands in cocktail, protect a considerable degree of challenge infection (Ridi *et al.*, 1993). In this study, immunization of mice with 0.2 mg of male adult worm



antigen through oral route one week before infection resulted in a 25% reduction in adult worms when compared with unimmunized infected mice. However, the difference is statistically insignificant ($P < 0.187$). The failure to confer significant protection with regard to the number of recovered worms here, may be due to the use of low immunogen concentration, or lack of booster dose. In the work of James and her coworkers (1985), a single intradermal immunization of mice with 0.1mg worm antigen in conjugation with Baccile Calmette Guerin (BCG) was not significantly protective (20%). According to their report lack of adjuvants in the vaccine does not seem to account for the low protection. However, the need for more booster dose and an increase in vaccine concentration cannot be discounted as responsible for the poor protection of the vaccine. Moreover, Fallon and Hagan (1996) have reported the role of IgG in the anti-fecundity effect of the parasite. Thus, association of high IgG level with protection could be in other parameters such as fecundity of adult worms.

This study demonstrated that the antibody levels of IgG and IgG1 in the sera of mice immunized with SAWA through oral route were significantly higher compared to the levels in the unimmunized infected mice before egg production by the parasite. The specific IgG antibody secreting response of mesenteric lymphocyte in immunized mice appeared earlier than in the unimmunized infected mice. These findings imply that mucosal immunization with SAWA would perhaps confer protection against subsequent challenge infections in schistosomiasis mansoni. In spite of this expectation, the immunization of mice with SAWA did not result in a significant reduction of recovered worms when compared with those from unimmunized infected controls. However, it should be noted that the number of adult worms alone may not be adequate to conclude about protection or the lack of it because it would be necessary to determine the viability of eggs and the nature and extent of granuloma formation in the liver.

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