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Evaluation of *Echinostoma liei* worm, metacercaria and redia antigens for schistosomiasis control

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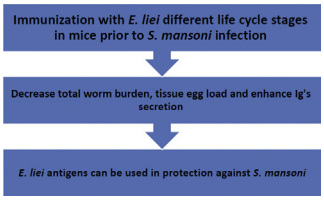
HIGHLIGHTS

- Antigens from*Echinostoma liei* were purified using CNBr-activated Sepharose column.
- Then used for immunization of mice prior to infection with *Schistosomiasis mansoni.*
- Worm burden, eggs and oogram count was significantly reduced and that was reflected in normalization of liver architecture.
- *E. liei* worm antigens induced the best reduction in worm burden and tissue egg load and acted as a good stimulator for Igs secretion.

GRAPHICAL ABSTRACT



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ABSTRACT

While chemotherepeutic drugs, such as praziquantel, oxamniquine and metrifonate, are currently considered safe and effective drugs for schistosomiasis treatment, reinfection occurs frequently after drug treatment. Thus, a vaccine is sought to provide long-term treatment. Antigens from worm, metacercaria and redia of *Echinostoma liei* (*E. liei*) were purified using CNBr-activated Sepharose column, then used for immunization of mice prior to infection with *Schistosomiasis mansoni*. Worm burden, hepatic and intestinal eggs and oogram count was significantly reduced and that was reflected in normalization of liver architecture. This referred to a significant increase in the tested immunoglobulin level (IgM, IgG1 and IgG2).

1. Introduction

Schistosomiasis or snail fever is one of the most significant parasitic diseases which have plagued humans since the time of ancient Egyptian and Chinese mummies (Nunn and Tapp, 2000). Its rank is high among parasitic diseases in terms of socioeconomic and public health importance in tropical and subtropical areas

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(Ferrari et al., 2003; Tan and Ahana, 2007).

Praziquental (PZQ), a highly active acylatedquinoline-pyrazine against all *Schistosoma* species, is now universally employed as recommended by the WHO for either individual or mass treatment (Anonymous, 2002; Doenhoff et al., 2009). It is revolutionary because it could be administered orally in a single dose and had very few unwanted side effects (Doenhoff and Pica, 2006). It is effective against cercariae, very young schistosomula and adult worms but does not kill 3–21 day old schistosomula (the migrating larvae) (Ross et al., 2002). Despite considerable efforts, the PZQ binding site on the schistosome surface and the precise mechanism of action are not yet known (Greenberg, 2005; Pica-Mattoccia et al., 2008). Concerns about reliance on a single drug to treat 200 million people include potential drug resistance (Keiser and Utzinger, 2007; Silva et al., 2004) and rapid reinfection rate, as people must be retreated on an annual or semiannual basis. Thus, identification of alternative schistosomiasis chemotherapies has become a high priority (Abdulla et al., 2007; Doenhoff et al., 2008).

Vaccination can be targeted towards either the prevention of infection or the reduction of parasite fecundity. Schistosome eggs being responsible for both pathology and transmission, a vaccine targeted toward parasite fecundity and egg viability appears, therefore, entirely relevant (Capron et al., 2002). There is potentially numerous and promising vaccine antigens from *Schistosomiasis mansoni* (Sm TSP-1, Sm TSP-2, Sm 29, Sm 23, Sm-p 80, Sm 14, Sm28-SGT, Sm28-TPI, Sm97 paramyosin and CT-COD) and, to a lesser extent, *Schistosoma haematobium*, only one vaccine, namely BILHVAX, or the 28-kDa GST from *S. haematobium*, has entered clinical trials (Capron et al., 2005).

The current *Schistosoma* vaccine candidates may prove not to be the most effective vaccine. It is important to identify new target antigens and to explore alternative vaccination strategies to improve vaccine efficacy. The available schistosome antigens and prototype vaccine formulations induce 40–50% protection in animals, at best, using the standard readouts of reduced worm burden or egg production and viability (McManus and Loukas, 2008).

Because larval *Echinostoma liei* has the upper hand when competing with *S. mansoni* miracidia to attack the snail host, this led to further study of any expected potential protective effect of purified antigens from different stages of *E. liei* against murine *S. mansoni* infection.

2. Materials and methods

2.1. Animals

Six to eight week old female albino CD1 mice (24 ± 2g) bred and maintained at the Schistosome Biological Supply Program, Theodor Bilharz Research Institute, Giza, Egypt (SBSP/TBRI). Mice were kept under standard laboratory care (21 °C, 45–55% humidity), filtered drinking water, 24% protein and 4% fat diet. Animal experiments have been carried out according to the internationally valid guidelines and ethical conditions (Nessim et al., 2000).

2.2. Parasites

2.2.1. S. mansoni cercariae

S. mansoni cercariae were provided by SBSP at TBRI. This strain has been moved through outbred mice and *Biomphalaria alexandrina* snails, cared for and maintained at SBSP/TBRI.

2.2.2. E. liei worms, metacercaria and redia

E. liei was routinely maintained in medical malacology laboratory at TBRI. Encysted metacercariae and rediae of *E. liei* were removed from the kidneys and pericardial cavities of experimentally infected *Biomphalaria glabrata* snails (El-Dafrawy, 1989; Fried et al., 1997). Adult *E. liei* worms were obtained from small intestine of mice experimentally infected with *E. liei*.

2.3. Infection

S. mansoni infection was performed by subcutaneous injection (s.c.) of 120 cercariae/mouse (Stirewalt and Dorsery, 1974).

2.4. Purification of E. liei antigens

Antigens prepared from metacercariae, rediae and worms of *E. liei* were purified using CNBr-activated Sepharose column according to Axen et al. (1983). Different tissues were individually homogenized in an iced glass tissue grinder and ultra-centrifuged at 18.000 rpm for 1 h at 4 °C. Antigens were eliminated from the supernatants by affinity chromatography (Carter and Colley, 1978). Antibody (Sigma Chem, Co., St. Louis, Mo) was linked to CNBr-activated sepharose 4B beads (Pharmacia fine chemicals, Piscataway, NJ) at ratio of 7 mg protein/ml of beads (Norden and Strand, 1984). Each supernatant was passed over a 1 ml column of antibody coupled beads. The non-adherent host antigen-free fraction was collected and dialyzed against phosphate buffered saline (PBS); 0.01 M, pH 7.2. The samples were sterilized by filtration through 0.45 μm filters (Nalgene Brand Product, Sybran corp.; Rochchester, NY) and the protein content was estimated using Bio-Rad kit (Bradford, 1976). All procedures were carried out at 4 °C.

2.5. Immunization with E. liei antigens

Different experimental mice groups were immunized with 3 types of *E. liei* antigens individually extracted from worms, metcercariae and rediae. Each antigen (100 μg/ml) was emulsified with Complete Freund adjuvant (CFA) and s.c injected as 1^{ry} immunization. Then, mice were boosted at 3rd and 4thweek (wk) post primary injection with only 50 μg/ml of antigens emulsified in incomplete Freund adjuvant (IFA).

2.6. Experimental design

A batch of 80 mice was divided into 8 groups (gp) (10 mice each), as follows:

gp A:	Normal healthy control group
gp B:	*S. mansoni* infected group
gp C:	Mice were immunized with *E. liei* worm antigen.
gp CI:	Mice were immunized with *E. liei* worm antigen. Three days later, all mice were infected with *S. mansoni* cercariae.
gp D:	Mice were immunized with *E. liei* metacercaria antigen.
gp DI:	Mice were immunized with *E. liei* metacercaria antigen. Three days later, all mice were infected with *S. mansoni* cercariae.
gp E:	Mice were immunized with *E. liei* redia antigen.
gp EI:	Mice were immunized with *E. liei* redia antigen. Three days later, all mice were infected with *S. mansoni* cercariae.