Characterization and Immunolocalization of *Schistosoma mansoni* Thioredoxin Glutathione Reductase on Different Life Cycle Stages of Infected Mice

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**ABSTRACT**

In the present study, polyclonal antibodies (pAb) were prepared against smTGR antigen and purified to demonstrate the presence of highly reactive epitopes on the different life cycle stages of *S. mansoni* and in different organs of infected mice. This will be performed in order to detect on which stage these antigen evince. In addition, the study aims to screen all possible organs for the localization of TGR antigen in an attempt to find out a suitable method for diagnosing the disease as early as possible. The results revealed that smTGR could be detected in various organs of infected mice and localized in all parasites life cycle stages except eggs using smTGR pAb.

**Key words:** *S. mansoni*, Immunolocalization, TGR, Polyclonal antibody.

**Introduction**

Schistosomiasis is a chronic and debilitating disease that is caused by parasitic trematode worms (*schistosomes*). It continues to threaten millions of people, particularly rural areas in the developing world (Chitsulo *et al*., 2000; Engels *et al*., 2002). Of the estimated 200 million infected people, more than half have symptoms only, while 20 million exhibit severe disease manifestations and 200,000 deaths each year with a further 700 million people at risk of infection (Rutitzky *et al*., 2005; Lea *et al*., 2008; Milligan and Jolly, 2011; Wilson *et al*., 2011).

Despite the availability of an effective drug and the implementation of successful control programs, the number of infected cases has not decreased during the last decades (Corsijsens *et al*., 2008). *S. mansoni* parasites survive in humans in part because of a set of antioxidant enzymes that continuously degrade reactive oxygen species produced by the host. A principal component of this defense system has been recently identified as TGR, a parasite-specific enzyme that combines the functions of two human counterparts, GR and TrxR, and as such this enzyme presents an attractive new target for anti-schistosomiasis drug development (Lea *et al*., 2008).

This study aims to purify the TGR secreted from the *S. mansoni*, preparation of anti-TGR polyclonal antibodies and study the localization pattern of TGR in different stages of the parasite.

The goal of immunocytochemistry, a combination of immunochemistry and morphology, is to define the cellular location of biochemically defined antigens. Immunocytochemical techniques can be applied to all types of cells. The post-embedding labelling is the most favourable procedure, thereby using an indirect immunostaining consisting of two successive immunoreactions: the antigen being first detected by the specific antibody and this primary antibody it self being recognized by a second antibody to which is attached a visual marker (Frugier and Crespi, 2006).

In making a choice between producing pAbs or mAbs, the desired application of the antibody and the time and money available for production should be considered. The fact that a polyclonal antiserum can be obtained within a short time (4-8 wk) with little financial investment favors its use, whereas it takes about 3 to 6 month to produce mAbs. Many research questions can be answered by using a polyclonal antiserum. MAbs are specific for an epitope, which can be essential in specific cases (Lipman *et al*., 2005).

There are critical points in immunocytochemistry: antibodies and fixation. The availability of antibodies is one of the main criteria for the use of immunocytochemical techniques, which are specific and show a high-affinity against a cellular antigen. Therefore, it is very important to verify that the antibody does not show any irrelevant cross reactivity. This may occur when the antigen of interest shares a common epitope with an apparently unrelated protein or the antigen used for immunisation was not pure enough. In the same time the ultimate aim of fixation is to freeze cell and tissue organization in a particular time frame so that every molecule in that cell or tissue remains in its original location during visualization. Chemical fixation using aldehydes is easy to perform and does not require special equipment. Formaldehyde and paraformaldehyde are the most frequently used aldehydes in immunocyto-chemistry (Frugier and Crespi, 2006).
Material and Methods

Experimental animals

New Zealand white male rabbits, weighing approximately 1.5 Kg that are about 3 months of age, were purchased from rabbit research unit (RRU), and laboratory bred male, Swiss albino mice strain, each weighing 18-20 g, were used in this study. The animal experiments were carried out according to the internationally valid guidelines and in an institution responsible for animal ethics [Schistosome Biological Supply program Unit at Theodor Bilharz Research Institute (SBSP/TBRI), Giza, Egypt] (Nessim and Demerdash, 2000).

Cercariae and infection

_S. mansoni_ cercariae were provided by SBSP/TBRI. Infection was performed by s.c. injection of 100 _S. mansoni_ cercariae to each mouse (Stirewalt and Dorsey, 1974).

Parasite antigen preparation

Mature _S. mansoni_ worms were recovered by porto-mesenteric perfusion of livers of _S. mansoni_ infected mice at 8-12 wk post-infection (PI). Deoxycholic acid (DOC) extracted material was performed from adult worms and the supernatant was stored at -80°C until use (Maggioli _et al._, 2004). Protein content of the prepared antigens was measured by the Bio Rad Protein assay kit (Bradford, 1976).

Purification of _S. mansoni_ TGR antigen

TGR was purified to homogeneity according to the method described by Rigobello and coworkers (1998), with some modifications. One hundred and eighty milliliters of DOC-extracted material (30 mg total protein) was submitted to ammonium sulfate fractionation According to Nowotny (1979) and (Harlow and Lane, 1988). Further purification of TGR antigen performed by 2’5’ ADP Sepharose 4B column chromatography (Hunt _et al._, 1983). And the purity of the produced protein was assayed by SDS-PAGE under reducing and non-reducing conditions (Harlow and Lane, 1988; Myers, 1995).

Assessment of reactivity of TGR of _S. mansoni_ by indirect ELISA

The antigenicity of the purified target antigen was tested by indirect ELISA technique, for serum samples of _S. mansoni_ infected-human and sera of patients infected with other parasites e.g., _Fasciola gigantica_, hydatid and hookworm. This method was performed, with some modifications from the original method of Engvall and Perlmann (1971).

Production of polyclonal antibodies

Immunization of rabbits for production of polyclonal antibodies

Rabbit anti-_S. mansoni_ TGR pAb was obtained by immunizing New Zealand white rabbits with _S. mansoni_ TGR antigen. 1 mg of _S. mansoni_ TGR antigen was given to each rabbit in entire course of immunization. The rabbits received priming dose as intramuscular injection at four sites [1 mg _S. mansoni_ TGR antigen mixed 1:1 in FCA (Sigma)]. Two booster doses were given, each was 0.5 mg antigen emulsified in FIA. The first boosting was 2 wk after priming dose. The following boosting doses were given at weekly intervals.

The rabbits were bled for collection of sera 1 wk later after a preliminary testing of titer by indirect ELISA. Rabbit sera that contain anti- _S. mansoni_ TGR pAb was fractionated and kept at -20°C.

Purification of rabbit anti-_S. mansoni_ TGR polyclonal antibodies

Rabbit IgG purification was based on sequential use of ammonium sulfate precipitation method (Nowotny 1979), caprylic acid purification method (Mckinney and Parkinson 1987), and DEAE-Sephadex A-50 ion exchange chromatography method (Sheehan and FitzGerald 1996). After purification, the protein content was estimated by Bio-Rad protein assay (Bradford 1976) and the purity of IgG was identified by SDS-PAGE (Laemmli 1970). Anti-TGR IgG were conjugated with horseradish peroxidase (HRP) using periodate method according to Tijssen and Kurstak (1984).
Immunolocalization of S. mansoni target antigens on the different organs of infected animals and different life cycle stages of S. mansoni

Collection of organs of naturally S. mansoni infected mice

Parts of livers, spleens, bile ducts, and gallbladders were taken from mice infected with the S. mansoni parasite and immediately fixed for 24 hr in 10% neutral buffered formalin solution and prepared in paraffin blocks in the Pathology Department in TBRI.

Agarose method

Different life cycle stages of S. mansoni (eggs, miracidiae, cercariae, schistosomula, and adult worm) were left individually for 24 hr in dechlorinated water until sedimented. Collection of eggs from murine intestines was performed according to the method of Liang and Kitiikoon (1980). Eggs were mixed with tissue-Tec to be stored briefly at -70°C until cryocut to get frozen section. The viable eggs were pipetted into a small petri dish (1.5 x 6 cm) and placed under ceiling illumination for hatching of miracidia. After collection, miracidia were extensively washed in 0.1 M PBS pH 7.3 and fixed in 0.05% formaldehyde solution for 3 hr at 4°C. After fixation, the miracidia washed with dist. H₂O and centrifuged for 3 min at 800 rpm. The supernatant was then drawn off, the sediment and cercariae, schistosomula and adult worm mixed with tissue-Tec to be stored briefly at -70°C until cryocut to get frozen section. The sediment was fixed in a mixture of equal vol. of 4% buffered glutaraldehyde and cacodylate 0.2 M for 1 hr and put in a small hole of about 3 mm in a layer of hardened 1.5% gelose followed by another liquefied gelose layer. The solidified gelose cubes containing the burred life cycle stages were manipulated as blocks. These blocks was refixed in 10% neutral buffered formalin and sent to the Pathology Department in TBRI for preparing paraffin blocks (Mansy, 2004).

Preparation of slides

Slides treated with 3-amino-propyl-triethoxy silane were used, paraffin blocks were cut by microtome at 5µ thickness, Sections were mounted on the glass slides, and the slides were incubated at 37°C overnight for accurate adhesion of the section to the slide. These slides were used instead of the ordinary albumenized slides to minimize staining artifacts and for better fixation of the sections on the slides.

Procedure for immunostaining

The staining method proceeded as follows:

Deparaffinization and rehydration of the sections

Slides were placed overnight in xylene, the slides were transferred into graded ethanol (100%, 95%, and 70%), 5 min each, then the slides were washed in dist. H₂O for 2 min, and the slides were washed in Tris buffer saline (TBS) for 5 min.

Blocking endogenous peroxidase activity

For blocking of the endogenous peroxidase, slides were incubated in a solution of 3% H₂O₂ in methanol for 30 min, this solution was freshly prepared, and then slides were washed in TBS.

Application of antibody

The polyclonal anti-rabbit-IgG, conjugated to HRP (diluted 1:250 TBS with 1% BSA), was then applied and incubated for 24 hr at room temperature. After another wash, the sections were incubated with diaminobenzidine tetra hydrochloride (DAB) substrate (Sigma) for 1 hr at room temperature in the dark. The reaction was stopped with dist. H₂O.

Dehydration of the sections

Slides were successively placed in 70%, 90%, 95%, and 100% alcohol each for 5 min, then washed three times in TBS.
Counter stain

The slides were immersed in Mayer's hematoxylin (Sigma) for 2 min then washed in tap water.

Mounting procedures

The cover slips were mounted using DPX (Purified Canada balsam).

Control Slides

Negative control slides were done within each section of immunohistochemical staining. As the sections was processed in the above-mentioned sequences but with replacement of the pAb anti-rabbit-IgG with PBS.

Immunofluorescent localization of schistosomal TGR antigen

According to EL-Dosoky et al. (1984), and De Water et al. (1988), microscope slides were dipped in cold acetone, air dried and then coated with bind saline (gamma-Methacryloxy-propyl-Trimethxy-Silane) (Pharmingen) which increase the fixation of sections on slides, overnight then slides were dried for 10 minute. The cryostat sections were fixed in cold acetone (-20 °C) for 10 min. and left to dry. Slides were washed with cold PBS, pH 7.8 and dried. Blocking of tissue sections was achieved by overlaying normal serum (10% normal goat serum in PBS/ 2% BSA) and incubating the slides for one hr at 37 °C then serum was drained but not washed. Fifty µl of TGR IgG pAb containing 50-µg protein in PBS/ 2% BSA was added and incubated overnight at 4 °C. The slides were washed 4 times by gently adding one drop of PBS to the top of section and lifting the slide so that the drop runs over and off the section and then left to dry. Fifty µl of anti rabbit IgG–fluorescein isothiocyanate conjugate (FITC) (Sigma) diluted 1:50 in PBS/ 2% BSA in 1:1000 Evans blue as a counter stain were added. Slides were incubated at room temperature in humid chamber for 1 hr in the dark. The slides were washed 4 times by gently adding one drop of PBS to the top of section and lifting the slide so that the drop runs over and off the section. They were then dried. One drop of entellen (Merck) was added and the section was covered with a cover slip. The reaction was read using fluorescent microscope (Reichert-Jung, New York, USA) and pictures were photographed by photostar (Reichert-Jung) automatic camera system.

Results

Purification of TGR antigen from DOC extracts of adult S. mansoni worms

The DOC extracts obtained from adult S. mansoni worms contains 8 mg/ml of total protein as measured by Bio-Rad Protein assay, while it was 0.72 mg/ml after purification with DEAE-sephadex A-50 ion exchange chromatography and 0.36 mg/ml following purification with 2’5’ ADP Sepharose 4B column chromatography. The antigen fractions obtained from DEAE Sephadex A-50 ion exchange chromatography is represented by a single peak with maximum OD value equal to 0.715 at fraction number 11. The purification by 2’5’ ADP Sepharose 4B column chromatography is represented by two peaks. The small peak with OD value equal to 0.982 at fraction number 6, and the large peak with OD equal to 1.492 at fraction number 16. The eluted protein fractions resulted from the different purifications methods was analyzed by 12.5% SDS-PAGE under reducing condition and showed only one band at 65 kDa which represents TGR as shown in lane 4 (Fig. 1).

Production of polyclonal antibody against TGR

Reactivity of polyclonal antibody against TGR

An increasing antibody level started 1 wk after the first booster dose. The OD reading recorded 0.35 before immunization, 0.82 priming dose, 1.86 1st boosters. Three days after the 2nd booster dose, immune sera gave a high titer against SmTGR antigen with OD of 2.97 at 1/250 dilution.

Specificity of polyclonal antibody against SmTGR antigen

Reactivity of anti-SmTGR pAb against SmTGR antigen and other parasite antigens (Fasciola, hookworm, hydatid and trichostrongylides) was determined by indirect ELISA. The produced anti-SmTGR pAb gave a strong reactivity to SmTGR Ag. The OD reading at 492 nm for S. mansoni was 2.84 compared to
0.262, 0.310, 0.206, and 0.281 for Fasciola, hookworm, hydatid, and trichostrongyloides, respectively.

Lane 1: Low MW standard.  
Lane 2: DOC extracts.  
Lane 3: Target antigen eluted from Sephadex A-50 exchange chromatography.  
Lane 4: Target antigen eluted from 2’5’ ADP Sepharose 4B column chromatography.

**Purification of rabbit anti-SmTGR polyclonal antibodies**

The IgG fraction of rabbit anti-S. mansoni pAb was purified using different purification steps summarized with its protein contents in (Table 2). The eluted anti-SmTGR IgG obtained by DEAE Sphadex A-50 ion exchange chromatography is represented by a single peak with maximum OD value 2.88 at fraction number 10.

<table>
<thead>
<tr>
<th>Purification Methods</th>
<th>Protein content</th>
</tr>
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<tbody>
<tr>
<td>1- Crude rabbit serum containing anti-S. mansoni pAb</td>
<td>7.1 mg/ml</td>
</tr>
<tr>
<td>2- 50% ammonium sulfate precipitation</td>
<td>4.2 mg/ml</td>
</tr>
<tr>
<td>3- 7% caprylic acid precipitation</td>
<td>3.1 mg/ml</td>
</tr>
<tr>
<td>4- Ion exchange chromatography</td>
<td>2.5 mg/ml</td>
</tr>
</tbody>
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**Characterization of anti-S. mansoni polyclonal antibodies by SDS-PAGE**

The precipitated proteins appeared as several bands. The purified pAb IgG was represented by H- and L-chain band at 53 and 31 kDa, respectively. The pAb appears free from other proteins (Fig. 2).

**Immunolocalization of target S. mansoni antigens on the different organs of naturally infected mice S. mansoni.**

Immunoperoxidase staining of different infected mice organs and different life cycle stages of S. mansoni infected mice organs were indicated by brown colour using the anti-SmTGR IgG pAb.
Lane 1: MW of standard protein.
Lane 2: Crude anti-\textit{S. mansoni} IgG pAb.
Lane 3: Precipitated proteins after 50\% ammonium sulfate treatment.
Lane 4: Purified IgG pAb after 7\% caprylic acid treatment.
Lane 5: Purified IgG pAb eluted from Sphadex A-50 exchange chromatography.

\textit{Liver}

After immunoastaining using anti-\textit{S. mansoni} IgG pAb, a positive peroxidase reaction was found in the sections of mice livers in the cytoplasm of the liver cells, histiocytes in the interlobular lesions and the necrotic foci of the hepatocytes and kupffer cells. The intensity of staining of kupffer cells was greater than those of infiltrating histiocytes (Fig. 4 A&B), compared to normal liver section of uninfected animal, or to the negative control which give negative peroxidase reaction (Fig. 3).

\textit{Spleen}

After immunostaining with anti-\textit{S. mansoni} IgG pAb, a positive peroxidase reaction as intracytoplasmic brownish granules was found in the lymphocytes of red pulp of the spleen (Fig. 6), in comparison to normal spleen section of uninfected animal, which give no peroxidase reaction or to the negative control that give negative peroxidase reaction (Fig. 5).

\textit{Gallbladder}

After immunostaining with anti-SmTGR IgG pAb, the mucosa is ulcerated and a positive peroxidase reaction was found in muscularis (Fig. 8), in comparison to normal gall bladder section of uninfected animal, or to the negative control, which give negative peroxidase reaction (Fig. 7).
Bile ducts

After immunostaining with anti-\textit{S. mansoni} IgG pAb, a positive peroxidase reaction was found in the cytoplasm of the cell lining of the bile duct (Fig. 9 B), in comparison to normal bile duct section of uninfected animal, which give no peroxidase reaction or to the negative control which give negative peroxidase reaction (Fig. 9 A).

Kidney

After immunostaining with anti-SmTGR IgG pAb, a positive peroxidase reaction was found in the cytoplasm of the epithelial cells lining the glomeruli and tubule of the kidney (Fig. 10 B), in comparison to normal kidney section of uninfected animal, or to the negative control which give negative peroxidase reaction (Fig. 10 A).

**Immunolocalization of target schistosomal antigen (TGR) in life cycle stages of \textit{S. mansoni}**

\textit{S. mansoni} eggs

No specific fluorescence reaction could be detected around \textit{S. mansoni} egg using anti-SmTGR pAb but the egg shell showed autofluorescence, which covered the internal structure (Fig. 11 B), in comparison to normal \textit{S. mansoni} egg (Fig. 11 A).

\textit{S. mansoni} miracidia

In an indirect immunofluorescence technique using anti-SmTGR pAb showed a positive immunofluorescence reaction, visualized as spots which were recognized around the miracidia (Fig. 12 B). In control experiments using PBS instead of pAbs, no fluorescence was observed (Fig. 12 A).

\textit{S. mansoni} cercariae

The tested anti-SmTGR pAb was found to be strongly reactive against cephalic and tail regions of \textit{S. mansoni} cercariae. An intense and sharply localized fluorescence appeared in the thin elongated primordial oesophagus and in the small terminal coecal bifurcation. Strong reaction was generally detectable in the oral and ventral suckers (Fig. 13 B). In control experiments using PBS instead of pAbs, no fluorescence was observed (Fig. 13 A).

\textit{S. mansoni} schistosomula

Significant fluorescence reaction was noted at the tegument, primordial esophagus and a strong reaction was generally detectable at the oral and ventral suckers (Fig. 14).

\textit{S. mansoni} adult worms

In an indirect immunofluorescence technique using anti-SmTGR pAb, a significant fluorescence reaction was detected at the tegument, oral and ventral suckers, in cross sections of adult worm, fluorescence reactions were mostly found in the parenchyma, and in the male worm pronounced fluorescence was observed in the gynaecophoric canal (Fig. 15).
Discussion

In the present study, the SmTGR antigen localized in different organs of *S. mansoni* infected mice and *S. mansoni* different life cycle stages using anti-SmTGR IgG pAbs. Our goal of the immunolocalization is to evaluate the potency of the postulant antigen as a diagnostic target antigen, also confirmed the previous studies which considered TGR as one of the most appealing drug targets against schistosomiasis (Angelucci *et al.*, 2010). All this is revealed by the mode of the distribution and concentration of TGR antigen in the organ and life stages, which reflect its importance for the parasite survival. For successful immunolocalization, the actual local concentration and specific localization of the antigen within a cell or organelle is of main importance.

For immunocytochemistry, it is necessary to perform negative controls (Frugier and Crespi, 2006); in the present study a double section as control in every organ were occupied, the infected organ without immunoperoxidase (negative control) and the uninfected organ with immunoperoxidase (normal control). In the life cycle stages of the parasite, the negative control prepared using PBS instead of pAbs.

After immunostaining using anti-SmTGR IgG pAb, a positive peroxidase reaction was found in the sections of mice livers in the cytoplasm of the liver cells, histiocytes in the interlobular lesions and the necrotic foci of the hepatocytes and Kupffer cells, compared to normal liver section of uninfected animal, or to the negative control which give negative peroxidase reaction. The intensity of staining of Kupffer cells was greater than those of infiltrating histiocytes indicating the intense concentration of the TGR antigen in these cells and the critical role of the SmTGR enzyme for parasite survival under oxidative and innate immunity stress.

These results agreed with a previous study (Hayashi *et al.*, 1999) where they stated that, the hepatic lymphocytes that had been stimulated with whole adult *Schistosoma* parasites (SWAP) presented by infected mice-derived Kupffer cells produced a huge amount of interleukin (IL-4), IL-13, and IL-5 as well as a little amount of interferon-gamma (IFN-γ) in response to immobilized anti-CD3 mAb. Kupffer cells from uninfected mice produced IL-6 and IL-10, but not IL-12 or IL-18, in response to SWAP stimulation and gained the potential to additionally produce IL-4 and IL-13 after the infection. These results suggested that prompt type 2 deviation in the liver after the infection might be due to the alteration of Kupffer cells that induces SWAP-mediated type 2-development of hepatic T cells.
In addition, in agreement to recent studies, which proved that, inflammatory responses upon liver injury, comprise resident as well as infiltrating immune cells. It is well known that innate immune cells are important triggers of hepatic inflammation; because the liver is selectively enriched in macrophages (Kupffer cells), natural killer (NK), and natural killer T cells (NKT) (Tacke et al., 2009). T-cell-mediated inflammation of the liver, four different types of T-helper cell responses have been described to influence various inflammatory processes in the liver. Th1 responses lead to classical activation of liver-resident macrophages such as Kupffer cells as well as recruitment of monocytes from the bloodstream, promoting a proinflammatory environment by secretion of IFN-γ, Tumor necrosis factor-alpha (TNF-α), and IL-12 (Hammerich et al., 2011).

The present study demonstrated a positive peroxidase reaction as intracytoplasmic brownish granules in the lymphocytes of red pulp of the infected mice spleen. In the gallbladder, the mucosa is ulcerated and a positive peroxidase reaction was found in muscularis. A positive peroxidase reaction was found in the cytoplasm of the cell lining of the bile duct and in the cytoplasm of the epithelial cells lining the glomeruli and tubule of the kidney compared to normal organs sections of uninfected animal, or to the negative control that give negative peroxidase reaction.

Our results agreed with El Amir and colleagues (2008), where they prepared pAb against Fasciola gigantica (F. gigantica) E/S products and purified it to demonstrate the presence of highly reactive epitopes on the different life cycle stages of F. gigantica as well as on the blood cells and different organs of naturally infected cattle. The produced result of this study were compared with the previous study despite the biological diversity among helminth parasites, they all induce similar immune responses in their hosts, characterized by a potent T-helper 2 (Th2) response and reduced Th1 response (MacDonald et al., 2002; Maizels et al., 2004), the helminth parasites F. hepatica and S. mansoni being both digenetic trematodes and are closely related organisms (Donnelly et al., 2008). However, Prxs are produced by more diverse helminth parasites such as nematodes, and their structure is very well conserved (the tr.

Although, as previously stated there is a high degree of similarity between the helminthes, this does not mean that there is no difference in between them. Otero et al. (2010) examined platyhelminth genomes and transcriptomes and found that all platyhelminth parasites (from classes Cestoda and Trematoda) conform to a biochemical scenario involving, exclusively, a selenium-dependent linked thioredoxin-glutathione system having TGR as a central redox hub. They identified TGR variants in Schistosoma species derived from a single gene, and demonstrated their expression. They also provided experimental evidence that alternative initiation of transcription and alternative transcript processing contribute to the generation of TGR variants in platyhelminth parasites.

In addition to the previous results, the present study demonstrated that SmTGR antigen localization is detected in S. mansoni different life cycle stages using anti-SmTGR IgG pAbs. There is no specific fluorescence reaction that could be detected around S. mansoni egg, as the egg shell showed autofluorescence, which covered the internal structure. Thus, it was expected that the presence of the egg in the host has no role in S. mansoni diagnosis using anti-SmTGR IgG pAbs, but this has no significant effect on the evaluation of the diagnostic potency of this enzyme where it is present sufficiently in other life stages. And this may be explain the results of the correlation between the S. bovis adult worm antigens (AWA) ELISA and the S. mansoni whole-worm egg antigen (WWE) ELISA of the sera from the patients with a definite diagnosis of schistosomiasis studied by Pardo et al. (2004). Their study exhibited in the S. bovis AWA ELISA, a higher mean OD than the sera used in the S. mansoni WWE ELISA (1.04 ± 0.347 versus 0.793 ± 0.244). This difference was statistically significant (P<0.001) thus they reported that, the fundamental advantage of using complex antigens from adult worms over using antigens from eggs is their greater facility and their high yield of antigenic material.

The absence of the specific fluorescence reaction around S. mansoni egg may be revealed that the Schistosoma eggs do not depend on the TGR as antioxidant. This hypothesis, do not conflict the result of previous studies which reported that the parasite depend on the antioxidants (other than TGR) in all stages of its life cycle. Where, Sayed et al. (2008) suggested that, the inhibition of TGR is through the modification of cysteine or selenocysteine residue in the protein. Dzik (2006) stated that, the enzyme system, Prxs, represents a major advance towards the understanding of how parasitic nematodes deal with both internal and environmental oxidative stress. Prxs exist as homodimers. They share the property of reducing hydrogen peroxide to water and alkyl hydroperoxides to the corresponding alcohols and classified into two families: the 1-Cys and 2-Cys Prxs according to the presence of one or two highly conserved cysteine residues. In the same field Boumis and coworkers (2011), study S. mansoni Trx and concluded that, it can reduce oxidized glutathione and is one of the
few defense proteins expressed in mature eggs and in the hatch fluid, thus confirming an important role in the parasite.

In the present study, the tested anti-SmTGR pAb was found to be strongly reactive against cephalic and tail regions of S. mansoni cercariae. An intense and sharply localized fluorescence appeared in the thin elongated primordial oesophagus and in the small terminal coecal bifurcation. Strong reaction was generally detectable in the oral and ventral suckers. A positive immunofluorescence reaction, visualized as spots were recognized around the miracidia by an indirect immunofluorescence technique using anti-SmTGR pAb where in control experiments using irrelevant pAbs, no fluorescence was observed. Significant fluorescence reaction was noted at the tegument, primordial esophagus and a strong reaction was generally detectable at the oral and ventral suckers in the schistosomula and highly significant in the adult worm, in the male worms pronounced fluorescence was observed in the gynaecophoric canal. In the adult worm, the heights' fluorescence appeared in the worm tegument. Most of these results agreed with El Amir and colleagues (2008), with consideration that, they studied the pAb against F. gigantica E/S product to demonstrate the presence of highly reactive epitopes on the different life cycle stages of F. gigantica.

The highly significant fluorescence reaction in the adult worm rather than in the schistosomula revealed by the previous researches and approve the demonstration of LoVerde and others (2004), that the expression of the schistosome antioxidant enzymes (Cu-Zn SOD; GPX) is developmentally regulated such that the lowest levels of gene expression (as measured by transcription) and enzyme specific activity were in the larval stages, the most susceptible to immune killing, and highest in adult worms, the least susceptible to immune elimination (Hong et al., 1992; Maizels et al., 1993; Mei and LoVerde 1995; 1997; LoVerde 1998; Carvalho-Queiroz, 2004). There are two paradigms that exist in schistosome immunology; the first is that the schistosomula stages are the most susceptible to immune killing and the second is that the adult stage, through evolution of defense mechanisms, can survive in the hostile host environment (Cook et al., 2004). In vitro studies have demonstrated that schistosomula are sensitive (95% killed) to oxidative killing, whereas adult worms exhibit much greater resistance (2% killed) to oxidative killing (Mkoji et al., 1988 a&b). The massiveness of the fluorescence in adult worm tegument reinforce Mei and LoVerde (1997) hypothesis that, if antioxidant enzymes were to protect the adult worms from ROS, the antioxidant enzymes should localize at the host-parasite interface where there immunolocalization studies demonstrated that SmCT-SOD, SmSP-SOD, and SmGPX localized to the tegument (host-parasite interface) of adult parasites supporting the notion that antioxidant enzymes are important in immune evasion by adult schistosome parasites. And this hypothesis ongoing for other antigen that responsible for protective the parasite against the host immunity, Sm14 is localized in tissues near the interfaces of parasite/host contact, such as the basal lamella of the tegument and the epithelium of the gut (Brito et al., 2002). In addition to the abundance of TGR antigen in the worm tegument we must remember that the tegmental antigens develop within 3 hr of host penetration by cercariae and thus their detection would diagnose active S. mansoni infection very early, reflect worm burden and proved to be an efficient immunodiagnostic tool for schistosomiasis (Hanallah et al., 2003). In Conclusion, the localization of SmTGR antigen, which is expressed in all developmental stages of the parasite and its distribution mode demonstrated in this study reveal several postulates, first, this antigen can play an important role in S. mansoni diagnosis, and it can be relied as a diagnostic target antigen. At the same time the researchers can depend on the diagnostic potency of this antigen in pursuance of the efficacy of any S. mansoni treatment thus it may contribute in control of schistosomiasis. The second, our results reinforce all previous studies that consider the TGR as one of the most appealing drug targets against schistosomiasis. The third, this study can be encouraging the studies that consider the TGR antigen as vaccine candidate.

References


