Schistosome Infection Stimulates Host CD4⁺ T Helper Cell and B-Cell Responses against a Novel Egg Antigen, Thioredoxin Peroxidase

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Egg granuloma formation during schistosome infections is mediated by CD4⁺ T helper (Th) cells sensitized to egg antigens; however, most of the relevant sensitizing egg antigens are still unknown. Here we show that schistosome thioredoxin peroxidase (TPx)-1 is a novel T- and B-cell egg antigen in schistosome-infected mice. CD4⁺ Th cell responses to fractionated egg components identified a significant response against a 26-kDa antigen; a partial amino acid sequence of this antigen was found to be identical to that of *Schistosoma mansoni* TPx-1. The native TPx-1 elicited significant proliferative responses as well as gamma interferon (IFN- γ), interleukin-2 (IL-2), IL-4, and IL-5 secretion in CD4⁺ cells from 8.5-week-infected CBA and C57BL/6 mice. By comparison, recombinant TPx-1 elicited a smaller, more type 1-polarized response, with significant production of IFN- γ and IL-2, less IL-5, and essentially no IL-4. In C57BL/6 mice the responses to TPx-1 were relatively more prominent than that directed against the major egg antigen, Sm-p40, whereas in CBA mice the reverse was true. B-cell responses were also monitored in infected C57BL/6, C3H, CBA, and BALB/c mice. All strains had significant antibody levels against the TPx-1 protein, but the most significant antibody production ensued following parasite oviposition. TPx-1 was localized in eggs and shown to be secreted by eggs. The identification of egg antigens is important to understand the specific basis of granuloma formation in schistosome infections and may prove to be useful in strategies to ameliorate pathological responses.

Schistosomiasis is a major public health concern in the tropics and subtropics, second only to malaria in importance. The disease is endemic in 74 countries and infects more than 200 million people, with 500 to 600 million at risk for infection (44). The immunopathological damage in schistosomiasis *mansoni* is due to granulomatous inflammation around parasite eggs in host liver and intestines, which may result in scarring, portal hypertension, hemorrhage, and death (6, 45). There is considerable variation in the severity of the disease in humans; of those infected, 20 million suffer severe consequences and 120 million are symptomatic. Several factors, including exposure, age, and a genetic predisposition to severe disease, have been suggested as cause for this variation (reviewed in reference 16). A similar variation in disease severity is also seen in laboratory infections of mice. For example, C3H and CBA mouse strains develop significantly larger egg granulomas than do the C57BL/6 (BL/6) strain (10, 18).

The formation of granulomas is a complex cellular hypersensitivity reaction that involves recruitment and activation of several inflammatory cells and the synthesis of extracellular matrix proteins, and it is under the control of numerous mediators, including cytokines. Granulomatous inflammation is known to be strictly dependent on CD4⁺ T helper (Th) cells specific for schistosome egg antigens (SEA) (22, 35). There now is strong evidence that granuloma formation can occur in an

environment dominated by CD4⁺ Th cells of the Th1 and/or Th2 type (12, 20, 42, 47, 48, 52). CD4⁺ Th cells become activated following specific recognition of cell-bound major histocompatibility complex class II molecules displaying selected SEA-derived peptides. However, the nature of most T-cell-sensitizing egg antigens and derived peptides remains largely unknown.

We have previously found that CBA mice display strong polyclonal T-cell responses against the major egg antigen Sm-p40, while in BL/6 mice this response is much weaker (3). Moreover, further analysis suggested that in CBA mice a significant proportion of the anti-SEA T-cell repertoire is directed against Sm-p40 (21, 23), whereas BL/6 mice develop a relatively more prominent response against *S. mansoni* phosphoenolpyruvate carboxykinase (Sm-PEPCK) (3, 4). These findings raise the possibility that different patterns of egg antigen recognition in mouse strains of different haplotypes influence the overall development of pathology.

To better understand the basis of the immune response, we have fractionated SEA into individual components to identify T-cell-stimulating antigens. In this study we show that there are strong polyclonal T- and B-cell responses in mice to a novel 26-kDa egg antigen, determined to be schistosome thioredoxin peroxidase (TPx)-1. TPx proteins are members of a novel class of antioxidant proteins, the peroxiredoxins, found in a wide variety of organisms and recently characterized by us for *Schistosoma mansoni* (30).

MATERIALS AND METHODS

Infection of mice. Six- to 8-week-old female BL/6 (*H-2^b*), CBA (*H-2^k*), C3H (*H-2^k*), and BALB/c (*H-2^d*) mice were purchased from the Jackson Laboratory (Bar Harbor, Maine). Mice were infected intraperitoneally with 70 cercariae of *S.*

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mansoni (Puerto Rico strain) obtained from infected *Biomphalaria glabrata* snails, provided by the Biomedical Research Institute, Rockville, Md. Infected, outbred Swiss-Webster mice, provided by the Biomedical Research Institute, were used to obtain adult worms and egg for Western blotting and immunohistochemistry.

Antigens. SEA was prepared as previously described (5) at the Biomedical Research Institute. The recombinant Sm-p40 antigen was prepared as previously described (21). Recombinant TPx-1 (rTPx-1) was expressed as a six-histidine-tagged protein in *Escherichia coli* and purified by nickel affinity chromatography as described previously (30).

CD4⁺ Th cell responses. CD4⁺ Th cells were isolated from mesenteric lymph node cells of mice 7.5 to 8.5 weeks after infection and pooled. The cells were purified by negative selection as previously described (21). CD4⁺ Th cells (1.5×10^5) were incubated in the presence of 4×10^5 irradiated syngeneic normal splenocytes (antigen-presenting cells [APC]) and the indicated antigens for a total of 114 h, and cell proliferation was assessed by tritiated-deoxythymidine ($[^3\text{H}]\text{dThd}$) incorporation as previously described (3). The experiments were repeated at least three times with similar results.

Determination of antigen-specific cytokine production. Purified CD4⁺ Th cells (10⁶) isolated from mesenteric lymph nodes of mice 7.5 to 8.5 weeks after infection were pooled and incubated for 24 to 48 h together with the indicated antigens in the presence of 4×10^6 irradiated syngeneic splenic APC. The cytokines gamma interferon (IFN- γ), interleukin-2 (IL-2), IL-4, and IL-5 were measured in culture supernatants by enzyme-linked immunosorbent assay (ELISA) as previously described (3). The experiments were repeated at least three times with similar results.

Preparation of native antigens. Native 26-kDa antigen was prepared from SEA by direct electroelution from a sodium dodecyl sulfate (SDS)–12.5 to 15% polyacrylamide gel as described previously (3).

Protein sequencing. SEA was fractionated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride membrane (Immobilin-P; Millipore) as described previously (3). Blots were stained with 0.1% Coomassie blue G in 50% methanol and destained with 10% acetic acid in 50% methanol. After washing blots with water and drying, the 26-kDa stimulatory fraction was excised for protein sequencing, which was carried out on an Applied Biosystems gas-phase sequencer (model 477A) at the Tufts Core Facility, Department of Physiology, Tufts University School of Medicine. The sequence was compared with sequences from known proteins by BLAST (Basic Logical Alignment Search Tool) programs from the National Center for Biotechnology Information.

Infection sera. Sera were obtained from 4 to 10 mice of the specified strain with infections of the indicated duration and pooled.

Determination of antigen-specific B-cell responses. A 96-well ELISA plate was coated with 62.5 ng of rTPx-1 per well and incubated overnight at room temperature. The wells were then washed three times with Nanopure distilled water (dH₂O). The wells were blocked with borate-buffered saline (BBS) (0.17 M H₃BO₄, 0.12 M NaCl, pH 8.5) plus 0.05% Tween 20, 1 mM EDTA, 0.25% gelatin, and 0.05% Na₂S₂O₃ for 1 h at room temperature prior to adding the sera obtained from BL/6, BALB/c, C3H, and CBA mice at different times during infection for 1 h at room temperature. After washing with dH₂O (three times), blocking solution was added to the wells and the plate was vortexed, incubated for 10 min at room temperature, and washed with dH₂O (three times). Secondary antibody, goat anti-mouse immunoglobulin G alkaline phosphatase-conjugated antibody (Jackson ImmunoResearch, West Grove, Pa.), was used at 1:1,000 and the plate was incubated for 1 h at room temperature. After washing with dH₂O (three times), the wells were developed using *p*-nitrophenyl phosphate (Pierce, Rockford, Ill.) in 0.05 M Na₂CO₃–0.05 mM MgCl₂ and read at 405 nm. ELISAs were performed in triplicate.

Western blotting. Adult worms were obtained by perfusion of infected mice (17). Eggs were isolated from the livers of infected mice as previously described (32). Antigens were prepared from adult worms and eggs by homogenization in a glass tissue homogenizer with a Teflon pestle in phosphate-buffered saline (PBS) and centrifuged at $14,000 \times g$. The supernatant was either used immediately or stored at -80°C . To prepare egg secretory products, eggs were isolated from the livers of 15 mice infected 7 weeks previously with 250 cercariae each. The livers were held overnight at 4°C prior to egg isolation. All of the eggs collected ($\sim 10^5$) were incubated in 2 ml of medium 199 (GIBCO/BRL, Grand Island, N.Y.) for 48 h at 37°C in 5% CO_2 . After the incubation period, the eggs were collected and the supernatant was removed. The supernatant containing the secreted egg products was concentrated by lyophilization to approximately 100 μl (83 $\mu\text{g/ml}$). At the end of the incubation period, very few eggs had hatched ($\ll 1\%$), and most eggs contained viable miracidia, evidenced by active miracidia inside the eggs. SDS-PAGE, blotting to polyvinylidene difluoride membranes, and Western detection were performed using standard techniques (43) on a

FIG. 1. Identification of a 26-kDa antigen recognized by CD4⁺ Th cells from schistosome-infected BL/6 mice and the N-terminal amino acid sequence obtained from the 26-kDa fraction. The eluted SDS-PAGE fraction containing the 26-kDa component (Fr. E22) (3) from 20 µg of SEA was examined for purity on a silver-stained SDS-15% polyacrylamide gel and is shown next to total SEA and molecular mass marker standards.

mini-PROTEAN II (Bio-Rad) apparatus. The membrane was blocked and then incubated with primary antibody overnight at 4°C. After washing twice for 15 min each in PBS, the blot was incubated for 3 h at room temperature with 1:3,000 goat anti-rabbit immunoglobulin G-alkaline phosphatase conjugate (Jackson ImmunoResearch), washed twice for 15 min each in PBS, and developed with detection buffer (100 mM Tris-HCl, 100 mM NaCl, pH 9.5) plus 0.33 mg of nitroblue tetrazolium per ml and 0.33 mg of 5-bromo-4-chloro-3-indolylphosphate per ml. Development was stopped by rinsing in dH₂O.

Immunohistochemistry. Polyclonal rabbit anti-rTPx-1 serum from a New Zealand White rabbit was affinity purified using a HiTrap *N*-hydroxysuccinimide-

FIG. 2. Proliferative response of CD4⁺ Th cells from BL/6 and CBA mice to the 26-kDa antigen. CD4⁺ Th cells were isolated from mesenteric lymph nodes of 8.5-week-infected mice. Culture conditions were as described in Materials and Methods. [³H]dThd incorporation was assessed by liquid scintillation spectroscopy. Data are expressed as the mean $\pm$ 1 standard deviation. Also shown for comparison are responses to Sm-p40 and SEA. Background radioactivity from cultures in the absence of antigen is subtracted. The same patterns of stimulation were observed when cells from 7.5-week-infected mice were used (not shown). The experiments were repeated at least three times with similar results.

FIG. 3. Cytokine production by $CD4^+$ Th cells from BL/6 and CBA mice stimulated with the 26-kDa antigen. $CD4^+$ Th cells were isolated from mesenteric lymph nodes of 8.5-week-infected mice and cultured with the indicated antigens in the presence of APC as described in Materials and Methods. The cytokines were measured in culture supernatants by ELISA. Data are expressed as means of triplicate determination $\pm$ 1 standard deviation. Also shown for comparison are responses to Sm-p40 and SEA. The experiments were repeated at least three times with similar results.

activated affinity column according to the instructions of the manufacturer (Amersham Pharmacia Biotech, Piscataway, N.J.). Briefly, rTPx-1 was covalently linked to the column, serum was passed over the column, the column was washed, the TPx-1-specific antibodies were eluted using 0.2 M glycine-HCl (pH 3.0), and the pH of the purified antibody solution was equilibrated to approximately neutral with 1 M Tris (pH 9.0).

Eggs were isolated as described above and fixed in 4% paraformaldehyde in PBS. The eggs were extensively washed in PBS plus 0.1% bovine serum albumin (BSA), 0.2% Triton X-100, and 0.2% NaN_3 . Fixed eggs were embedded in Tissue-Tek OCT embedding medium. Five-micrometer egg sections were cut and affixed to glass slides previously coated with poly-L-lysine. The sections were blocked with PBS plus 0.5% BSA and 5% goat serum for 1 h at room temperature. The sections were incubated overnight at 4°C with either affinity-purified or preimmune serum at 1:500. The slides were washed twice for 15 min each with gentle agitation in PBS plus 0.5% BSA. All subsequent steps were performed in the dark. The slides were incubated for 1 h at room temperature with 1:500 goat anti-rabbit Cy3-conjugated antibody (Jackson ImmunoResearch). The slides were again washed as described above before addition of Gel/Mount (Biomed, Foster City, Calif.) mounting medium and coverslips. Nuclear counterstaining was achieved by flooding the slides with a 250-ng/ml solution of 4',6-diamidino-2-phenylindole (DAPI) (Sigma, St. Louis, Mo.) for 1 h at room temperature.

RESULTS

Identification of a 26-kDa antigen in SEA that stimulates mouse $CD4^+$ Th cells. An initial characterization of the relative immunogenicity of egg antigens in schistosome-infected

BL/6 mice identified a number of protein components, including a 26-kDa fraction (3). In order to better characterize individual proteins, SEA was fractionated by SDS-PAGE on a 15% gel. The 26-kDa fraction was isolated from the gel and the N-terminal peptide sequence was determined as described above (Fig. 1). The amino-terminal peptide yielded a sequence, VLLPNRPAPEFKGQAVINGE, that is an exact match to amino acids 2 to 21 of the predicted sequence of TPx-1 (GenBank accession number AF121199) of *S. mansoni* (30). TPx is a member of a novel class of antioxidants recently described for a wide variety of organisms (8, 37), which exhibit hydroperoxide and alkyl hydroperoxide reductase activities and are thought to be involved in oxidative stress reduction mechanisms (2, 9, 26, 31, 40). We have shown that TPx protein and activity are abundant in adult worms, suggesting that they play an important role in preventing damage from reactive oxygen compounds (30).

Polyclonal Th cell responses to the 26-kDa antigen. To determine its relative immunogenicity, proliferative and cytokine responses to the native 26-kDa antigen were measured in polyclonal $CD4^+$ Th cells from infected mice. Figure 2 shows that in BL/6 mice, low concentrations of the 26-kDa protein elicited

FIG. 4. Proliferative responses of CD4⁺ Th cells from infected BL/6 and CBA mice to rTPx-1. CD4⁺ Th cells were isolated from mesenteric lymph nodes of 8.5-week-infected mice and incubated with the indicated antigens in the presence of APC as described in Materials and Methods. [³H]dThd incorporation was assessed by liquid scintillation spectroscopy. Also shown for comparison are responses to Sm-p40 and SEA. Background radioactivity from cultures in the absence of antigen is subtracted. The same patterns of stimulation were observed when cells from 8-week-infected mice were used (not shown). The experiments were repeated at least three times with similar results.

a significant dose-dependent proliferative response compared to unfractionated SEA. The response to the 26-kDa protein was significantly stronger than the response to the Sm-p40 antigen, which is a major egg immunogen in mice of the *H-2^k* haplotype (7, 21, 23). In contrast, in the CBA (*H-2^k*) mouse, the proliferative response to the 26-kDa protein was significantly less than the response to Sm-p40 (Fig. 2).

Examination of culture supernatants from CD4⁺ Th cells obtained from 8.5-week-infected BL/6 mice showed that the 26-kDa antigen elicited significant secretion of IFN- γ and IL-5 but smaller amounts of IL-2 and IL-4 (Fig. 3). For each cytokine tested, the response to the 26-kDa antigen was greater than the response to Sm-p40. However, in CBA mice, the 26-kDa antigen elicited less IFN- γ and IL-2 but more IL-4 and IL-5 than the Sm-p40 antigen.

Polyclonal Th cell responses to rTPx-1. In order to further characterize the antigenicity of the 26-kDa antigen, Th cell responses to rTPx-1 were investigated. rTPx-1 was expressed in *E. coli* and purified as a six-histidine-tagged fusion protein by nickel affinity chromatography. Figure 4 shows that a weak but significant proliferative response to rTPx-1 was seen in CD4⁺ Th cells from both BL/6 and CBA mice.

Examination of culture supernatants for cytokine levels revealed that stimulation with rTPx-1 elicited in both BL/6 and CBA mice significant IFN- γ , lower levels of IL-2 little IL-5, and virtually no IL-4 production (Fig. 5). Cytokine secretion observed with rTPx-1 was similar to secretion stimulated by Sm-p40. Significant IL-4 production was elicited in response to SEA in both mouse strains.

B-cell responses to recombinant TPx-1. To ascertain its relative antigenicity as a B-cell antigen, we measured antibody responses to rTPx-1 in BL/6 and C3H mice at 4, 5, 6, 7, 9, and 15 weeks postinfection and in CBA and BALB/c mice at 9 and 15 weeks after infection (not shown). A significant antibody response closely followed egg deposition, which begins at 5 to 6 weeks, and rapidly increased to a maximum at 9 weeks, followed by a significant decrease at week 15 (Fig. 6).

TPx-1 in eggs and secreted by eggs. In order to determine the relative amount of TPx-1 in eggs and whether eggs secrete TPx-1, total adult male and female homogenates and SEA along with egg in vitro culture supernatant were analyzed by SDS-PAGE and Western blotting. SDS-PAGE analysis of SEA and egg secretory products clearly shows that the two samples are different (Fig. 7, lanes 3 and 4). SEA has a protein banding pattern very similar to that of adult male and female homogenates. Egg secretory products are enriched in two proteins of approximately 23 and 33 kDa, neither of which is TPx-1, with much smaller amounts of other proteins. The identities of these two enriched proteins are unknown.

Western blotting showed that TPx-1 was present in all homogenates and egg secretory products (Fig. 7, lanes 5 to 8). The apparent molecular mass of the major isoform of TPx-1 in each sample varied as follows: egg secretory = SEA > male > female. Recombinant TPx-1 has an apparent mass of 29.8 kDa, greater than that of the native protein due to the presence of the 3.4-kDa six-histidine fusion peptide, the major TPx-1 protein bands are 25.1 kDa in males, 23.8 kDa in females, and approximately 26 kDa in eggs (data not shown). The differences in molecular masses and in the broadness of the egg and egg secretory product bands may reflect stage-specific glycosylation of the TPx-1 protein.

Immunohistochemical localization of TPx-1 in eggs. Affinity-purified antibodies were used to localize TPx-1 in schistosome eggs (Fig. 8). TPx-1 staining (Cy-3-conjugated secondary antibody) is red and is seen in the region between the miracidium and the eggshell, which corresponds to von Lichtenberg's envelope (39). We see no evidence of TPx-1 staining inside miracidia. In Fig. 8, autofluorescence of the eggshell is bright greenish yellow and nuclei of the miracidium are visualized by counterstaining with DAPI (blue fluorescence). No staining of the von Lichtenberg's envelope was seen with preimmune serum or DAPI staining alone (data not shown).

DISCUSSION

The pathological consequences of schistosome infection are the result of parasite eggs trapped in host tissue and the ensuing granulomatous reaction. The development of the liver granuloma is dependent on CD4⁺ Th cell responses to secreted egg antigens. Only a limited number of schistosome egg antigens have been described. The dominant egg antigen, Sm-p40, elicits a strong Th-1-type response in mice genetically predisposed to developing large granulomas (7, 22, 23). In contrast, mice predisposed to developing attenuated granulomatous responses, e.g., BL/6 mice, have weaker responses to Sm-p40 and recognize a wide range of egg antigens, with a prominent lymphoproliferative response directed against a 62-kDa component determined to be Sm-PEPCK (3). This study

FIG. 5. Cytokine production by $CD4^+$ Th cells from BL/6 and CBA mice stimulated with rTPX-1. $CD4^+$ Th cells were isolated from mesenteric lymph nodes of 8.5-week-infected mice and cultured with the indicated antigens in the presence of APC as described in Materials and Methods. The cytokines were measured in culture supernatants by ELISA. Data are expressed as means of triplicate determinations $\pm$ 1 standard deviation. Also shown for comparison are responses to Sm-p40 and SEA. The experiments were repeated at least three times with similar results.

has identified and characterized the immune response to another egg antigen, the 26-kDa TPx. We have recently shown that TPx is present in adult worms and likely plays a significant role in defense against oxygen radicals (30).

The native 26-kDa TPx elicited a relatively stronger proliferative response in BL/6 mice than in CBA mice, a situation similar to that with Sm-PEPCK (3) and unlike that observed with Sm-p40, which is a potent immunogen in CBA mice (21). Moreover, while Sm-p40 stimulates dominant Th-1 cytokine production, 26-kDa TPx elicits a more balanced Th-1/Th-2 cytokine response.

rTPx-1 elicited in both mouse strains a predominantly Th-1-type cytokine response, with high levels of IFN- γ and substantial IL-2 but less IL-5 and essentially no IL-4. The proliferative response in both BL/6 and CBA mice to rTPx-1 was significantly lower than that to the native antigen. A similar type of immune response to recombinant antigen has been noted by us in the case of Sm-PEPCK (4) and by others (49). One obvious difference between native and recombinant proteins produced in *E. coli* that may account for the differential cytokine production is that native proteins may be glycosylated whereas recombinant proteins are not. In particular, schisto-

somal egg components are known to be heavily glycosylated (13). The size of native TPx protein, 26 kDa, determined here is significantly larger than the predicted size of 21 kDa. This suggests posttranslational modification of the protein, although we do not know what, if any, glycosylation occurs. There is no endoplasmic reticulum-Golgi-targeting sequence in the peptide predicted from the TPx-1 cDNA. However, cytoplasmic glycosylation through O-linked *N*-acetylglucosamine of many membrane-associated and secreted glycoproteins derived from adult schistosomes has been shown to occur (41), although the level of glycosylation of any particular protein is not known yet. The results from Western blotting showing major isoforms of different molecular masses in adults and eggs suggests some sort of posttranslational modification.

The immune responses were examined in $CD4^+$ T cells obtained from mesenteric lymph nodes. In our view, mesenteric lymph node cells best represent the immune response to eggs in schistosome infections. The pattern and magnitude of immune response to schistosome antigens may differ depending on whether mesenteric, mediastinal, hepatic, and/or splenic lymphocytes are examined. Differences in T-cell responses from different compartments must await future studies.

FIG. 6. B-cell response to schistosome TPx-1. Sera were obtained from mice at the times indicated after infection. Anti-TPx-1 antibodies in the sera were measured by ELISA using microtiter plates coated with recombinant *S. mansoni* TPx-1. Data are expressed as means of triplicate determinations $\pm$ standard error. Sera were from BL/6 mice (closed bars) and C3H mice (open bars). OD, optical density.

The antibody response to TPx-1 closely followed parasite oviposition and rapidly increased to maximal levels at 9 weeks of infection (Fig. 6). Of interest is the finding that all mouse strains tested have serum antibodies against the recombinant, nonglycosylated form of TPx-1, which suggests that a significant portion of the antibody response is directed against peptide determinants. This is the first demonstration that TPx-1 is a target of an antibody response during schistosome infections. The antibody response to TPx-1 differs from that reported for Sm-p40/p38, in which IgG was first detected at 8 weeks of infection, with a gradual increase to a maximum at 16 weeks (11). Detailed characterization of the antibody responses in relation to subclasses or glycosylation of antigens must await future studies.

Western blotting showed that eggs have a higher level of TPx-1 protein than both adult male and adult female worms and that TPx-1 is present in egg secretory products. The egg secretory products that we have collected represent a unique subset of egg proteins. TPx-1, although present and enriched in this fraction, is not a major component. Identification of the major protein components of this fraction remains to be completed. Localization of TPx-1 to von Lichtenberg's envelope is most interesting. von Lichtenberg's envelope is a syncytium that completely surrounds the developing embryo and mature miracidium. It is thought to act as a barrier between the embryo-miracidium and the host extracellular fluid, preventing passive diffusion into or out of the egg (39). It is thought to be active in the uptake of nutrients for the developing embryo and secretion of its waste material. Our results suggest that von Lichtenberg's envelope is a source of secreted egg antigens, including TPx-1.

TPxs are antioxidant enzymes found in a wide variety of species, including helminths, protozoa, bacteria, fungi, vertebrates, and plants (37, 38). We have shown that TPx activity in adult *S. mansoni* is expressed at levels comparable to those of other antioxidant proteins (30). In other parasites, TPx has been found in secretory and excretory products (36, 51), located at the surface of parasites (34, 53), and as the target of host immune responses during natural or experimental infections (19, 29, 46, 50). Immunization with TPx has conferred

FIG. 7. Identification of TPx-1 protein in egg homogenates and secretory products. SDS-16% polyacrylamide gels were Coomassie blue stained (lanes 1 to 4) or transferred to membranes and probed with affinity-purified anti-*S. mansoni* TPx-1 antibodies (lanes 5 to 8). Lanes 1 and 5, 10 μ g of adult male homogenate; lanes 2 and 6, 10 μ g of adult female homogenate; lanes 3 and 7, 10 μ g of egg homogenate; lanes 4 and 8, 3.75 μ g of cultured egg secretory products. Protein size standards are shown on the left of each gel in kilodaltons. The figure was produced from scanned gels or membranes using Adobe Illustrator.

protective immune responses against *Leishmania major* (51) and *Entamoeba histolytica* (47) infections.

The likely function of TPx-1 in eggs is protection from damage due to immune-generated toxic oxygen compounds. Oxygen radicals are produced by granulomatous inflammatory cells (1), and in vitro studies have documented that eosinophils are capable of destroying eggs through a process that is antibody dependent (24, 25) and requires the release of reactive oxygen molecules (28). Schistosomes contain enzymes capable of neutralizing these oxygen radicals (reviewed in reference 33), but little work has been done to characterize their role in the egg. If TPx-1 enzyme activity plays a role in defense against reactive oxygen compounds, both enzyme cofactors, thioredoxin and thioredoxin reductase, should be present in the eggs

FIG. 8. Immunohistochemical localization of TPx-1 in schistosome eggs. A fluorescence micrograph of a 5- μ g egg cryosection is shown. Green fluorescence surrounding the egg is due to autofluorescence of the eggshell. Orange-red Cy3-labeled secondary antibody fluorescence is found in the von Lichtenberg envelope surrounding the miracidium and underneath the eggshell. DAPI-stained nuclei of the miracidium appear blue. The image is an overlay of three separate color images created in Adobe Photoshop. No specific Cy3 fluorescence was seen when preimmune serum was used for staining (not shown).

as well. We have detected thioredoxin and thioredoxin reductase mRNAs and thioredoxin reductase protein in schistosome eggs (D. J. Botkin, H. M. Alger, and D. L. Williams, unpublished data). Whether eggs also secrete these proteins remains to be determined.

Results from experimental infections in immunodeficient mice suggest that schistosome eggs require the host granulomatous response to facilitate passage across host tissue into the bowel lumen for discharge in feces (14, 15). Studies with human immunodeficiency virus- and schistosome-coinfected humans support a similar function of the granuloma in human infections (27). While the parasite may require this immune response for continuation of its life cycle, it must also withstand immune destruction within the granuloma by activated eosinophils and other immune cells. Hence, the presence of antioxidant proteins, such as TPx-1, in the egg may be essential to prevent oxidative destruction.

In this study we have identified schistosome TPx-1 as a novel egg antigen. Both native and recombinant TPx-1 stimulate a significant CD4⁺ Th cell proliferation in BL/6 and CBA mice. In all mouse strains tested there was also a strong B-cell response timed with egg production of the parasite and a switch to a Th2-type response. We have shown that TPx-1 in eggs is localized to the von Lichtenberg's envelope surrounding the miracidium and is secreted by eggs. Identification of important parasite T-cell antigens could aid the development of therapies to manage cellular responses and modulate pathology associated with schistosome infections.

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