Cloning and Characterization of a *Schistosoma mansoni* cDNA Clone with a Specific Antigenic Expression during Development in a Vertebrate Host

Arlete Martins Coelho-Castelo, Vanderlei Rodrigues+

Laboratório de Biologia Molecular de Parasitas, Departamento de Parasitologia, Microbiologia e Imunologia, Faculdade de Medicina de Ribeirão Preto, USP, Av. Bandeirantes 3900, 14049-900 Ribeirão Preto, SP, Brasil

Approximately 2.0 x 10 cDNA clones of an Schistosoma mansoni *λgt11 cDNA library were screened in duplicate with serum from infected mice corresponding to distinct phases of infection. A cDNA clone (7/1) was isolated and recognized only by seven week serum. The clone was subcloned in pGEX-2T and Western-blot studies showed a specific antigenic expression confirming that only serum from the chronic phase is capable of recognizing this antigen. Dot-hybridization with RNA from different developmental phases of the parasite showed that the corresponding 7/1 RNA is expressed in all phases of parasite development in vertebrate hosts.*

Key words: *Schistosoma mansoni* - antigen specific expression - cDNA - cloning

Schistosomiasis caused by *Schistosoma mansoni* is a chronic and debilitanting disease affecting 8 to 10 million people in Brazil (Lambertucci & Barravieira 1994). Chemotherapy is currently the main tool in the control of this disease and the recent development of vaccines represents a new strategy in the control of transmission (Balloul et al. 1987, Goudot-Crozel et al. 1989, Shoemaker et al. 1992, Tendler et al. 1995). During development in vertebrate hosts the parasite follows a complex route of migration to reach the porta-hepatic system. This process is associated with important structural, physiological and biochemical adaptations with differential antigen expression. In vertebrate hosts, about 3 hr after skin penetration cercariae transform into schistosomula which can be detected in the lungs during the first week of infection. Between the 4th and 5th week of infection the parasites of opposite sex become coupled and start oviposition in the porta-hepatic system (Miller & Wilson 1978, Wilson & Coulson 1986). The identification and characterization of phase-specific antigen expression may provide a better understanding of parasite biology and of the host-parasite interactions.

In the present study we have identified and characterized a cDNA clone with specific antigenic expression during *S. mansoni* development in a vertebrate host.

MATERIALS AND METHODS

Animal serum - Swiss mice were infected with 250 cercariae of *S. mansoni* LE strain by the method of Smithers and Terry (1965). Mouse serum samples were collected weekly for seven weeks after infection. Pooled serum from each week was purified by precipitation with 45% amonium sulfate, dialyzed against 10 mM PBS, pH 7.4, and used to immunoscreen a cDNA library.

Library screening - Duplicate filters of a recombinant λgt11 cDNA library were immunoscreened with serum from the 1st and 7th; 2nd and 7th; and 3rd and 7th weeks post-infection. Reactive clones were detected with goat anti-mouse IgG alkaline phosphatase conjugate at 1/5000 dilution and stored at 4ºC in SM buffer (20 mM Tris-HCl, pH 7.4, 10 mM MgSO4; 100 mM NaCl and 2% gelatin) containing 0.3% chloroform.

Dot-hybridization - 20 μg of mRNA from different phases of the parasite life cycle were denatured and dot-blotted onto nylon membranes and covalently linked to the filter using UV exposure. The filters were hybridized with cDNA 7/1 labeled with biotin according to manufacturer instructions (Photogene).

Expression in Escherichia coli and partial purification of expressed protein - The insert 7/1 was excised with EcoRI, purified out of gels and ligated into the phosphatase treated EcoRI site of the expression vector pGEX-2T (Smith & Johnson 1988). The recombinant plasmids were trans-

This work was supported by grants from FAPESP, CNPq and FINEP.

+Corresponding author. Fax: + 55-16-633.6631. E-mail: vrodrigu@famrp.usp.br

Received 16 April 1997
Accepted 30 June 1997

formed into *E. coli DH5* α (Hanahan 1983) and positive clones were grown in LB supplemented with 150 μg/ml ampicillin. Following induction with 1 mM IPTG, the pelleted bacteria were resuspended in MTPBS (15 mM NaCl, 160 mM Na2HPO4, 40 mM NaH2PO4, and 0.5 mM PMSF) and lysed by sonication. The bacterial lysate was made up to 1% (w/v) in Triton X-100 and after centrifugation at 10,000 *g* the supernatant was incubated with 30 mg glutathione-agarose beads for 30 min at room temperature. The glutathione-agarose beads were washed with PBS-Triton and incubated with thrombin at 25 units/ml according to the method of Smith and Johnson (1988). Beads were pelleted by centrifugation and the clarified supernatant was analyzed by SDS-PAGE under reducing conditions (Laemmli 1970)

Western blot analysis of recombinant antigen expression - For Western blot analysis, recombinant protein was electrophoresed as above and then transferred to nitrocellulose membranes. Blot was probed by overnight incubation with serum from different weeks post-infection and then incubated with goat anti-mouse IgG alkaline phosphatase conjugate and developed with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Gibco).

RESULTS

Immunoscreening of the λgt11 S. mansoni library and analysis of clones - Approximately 2 x 10^5 plaques from the λgt11 library of *S. mansoni* were screened for expression of antigenic determinants with immune mouse antiserum against *S. mansoni* using duplicate filters. We isolated 1 recombinant clone (7/1) recognized by 7th week serum but not by 1st week serum, 5 clones (7/2 a to e) recognized by 2nd week serum but not by 7th week serum, and 6 clones (7/6 a to f) recognized by 3rd week serum but not by 7th week serum. The cDNAs clones were isolated from recombinant phage and cut with the restriction enzyme EcoRI in order to determine the size of the insert fragments (Fig. 1).

The 7/1 cDNA clone was subcloned on pGEX-2T expression vector and the fusion protein recovered from bacterial lysate on glutathione-agarose beads (Fig. 2A). It was impossible to get the recombinant antigen without the GST portion after treatment with thrombin (Fig. 2B) probabily this impossibility may be explained due to conformation acquired by the complex (fusion protein) became the thrombin site inaccessible for the cleavage as described before in others situations (Guan & Dixon 1991).

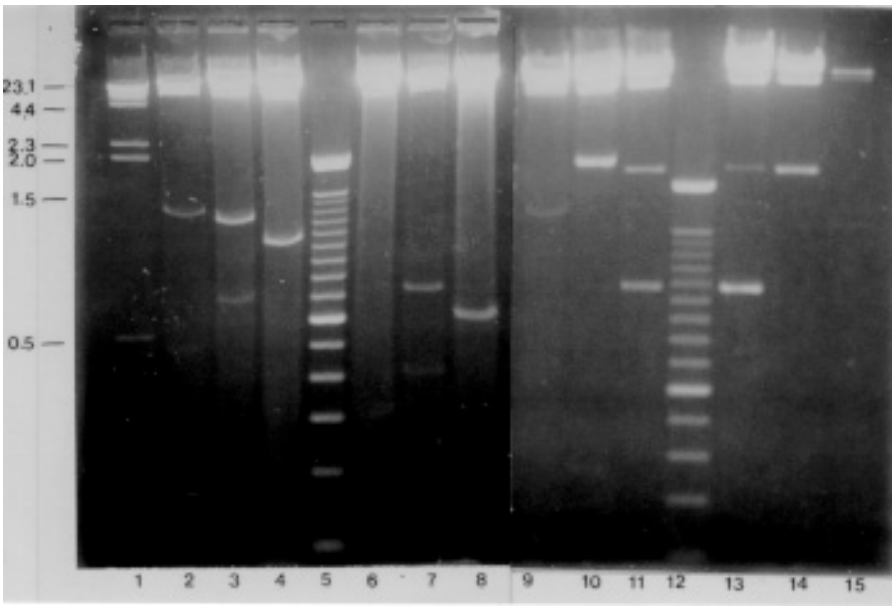

Fig. 1: characterization of *Schistosoma mansoni* cDNA clones. Recombinants clones of λgt 11 were digested with EcoRI for 2 hr at 37ºC and the size of inserts was visualized by electrophoresis on 1.5% agarose gel stained with ethidium bromide. Lane 1: λDNA-Hind III. Lane 2: clone 7/1. Lane 3: clone 7/2a. Lane 4: clone 7/2b. Lane 5: 100 bp DNA ladder. Lane 6: clone 7/2c. Lane 7: clone 7/2d. Lane 8: clone 7/2e. Lane 9: clone 7/3a. Lane 10: clone 7/3b. Lane 11: clone 7/3c. Lane 12: 100 bp DNA ladder. Lane 13: clone 7/3d. Lane 14: clone 7/3e. Lane 15: clone 7/3f.

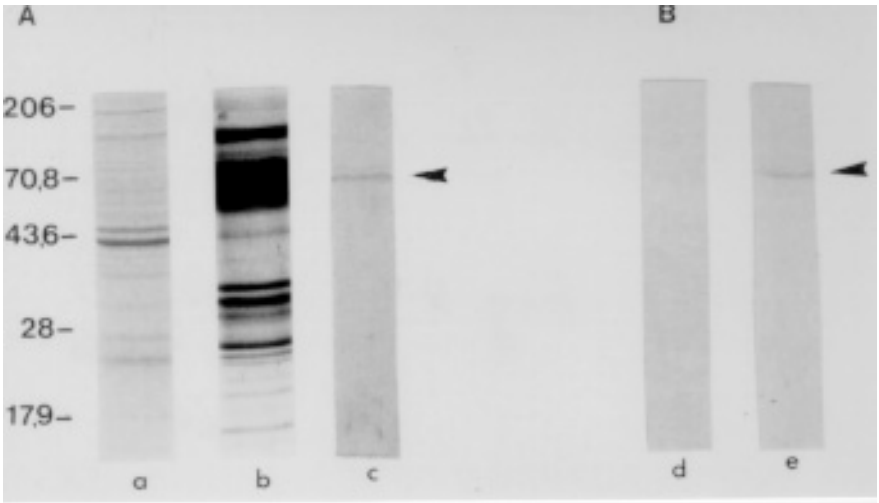

Fig. 2: SDS-PAGE of expression and purification of the pGEX 2T-7/1 recombinant clone. (A) Lysates of DH 5 α transformed with pGEX 2T-7/1 without induction (lane a). After 60 min of induction (lane b). After purification with glutathione-agarose beads (lane c). (B). Supernatant of the incubation with glutathione-agarose beads and after thrombin cleavage (lane d). Material bound to glutathione-agarose beads (lane e). The position and sizes (kDa) of molecular weight markers are indicated.

Immunogenicity of the 7/1 clone during schistosome infection - It was of interest to determine whether clone 7/1 showed specific expression during *S. mansoni* infection. Western blot of induced 7/1 clone in pGEX-2T showed that the antigen produced as a fusion protein with GST reacted only with serum from mice with 6 and 7 weeks of infection (Fig. 3). GST alone was not recognized by either serum (results not shown).

Analysis of mRNA expression related to 7/1 cDNA - In order to examine 7/1 mRNA expression, equal amounts of total RNA from miracidia, cercariae, eggs, schistosomula, and male and female adult worms were blotted on nitrocellulose filters and hybridized to 7/1 cDNA. The hybridization signal was detected with RNA from schistosomula and from male and female adult worms (Fig. 4A, tracks c, d, e, f) and was not detected with RNA from miracidia, cercariae or eggs (Fig. 4A, tracks a, b, g). As a control, the RNA samples were also hybridized with actin (Fig. 4B).

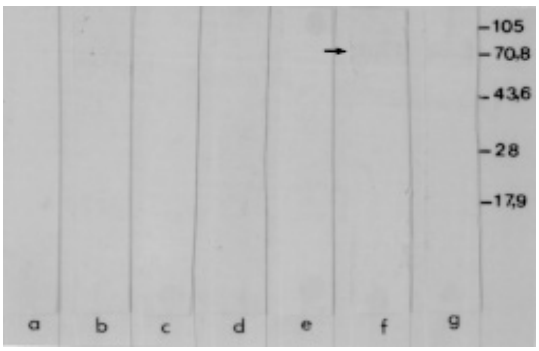

Fig. 3: Western blot analysis of the 7/1 fusion protein. Lysates of the induced pGEX 2T-7/1 clone were separated by SDS-PAGE, Western blotted and probed with the following antisera. Lane a: 1st week serum. Lane b: 2nd week serum. Lane c: 3rd week serum. Lane d: 4th week serum. Lane e: 5th week serum. Lane f: 6th week serum. Lane g: 7th week serum. Molecular weight markers (kDa) are indicated on the right.

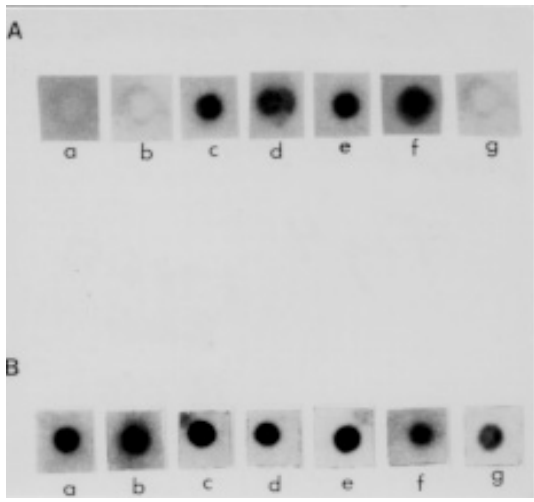

Fig. 4: Dot blot analysis of the mRNA 7/1 clone expression. Total RNA of the miracidia (lane a). Cercariae (lane b), schistosomula (lane c), male (lane d), female (lane e), worm pair (lane f). Eggs (lane g) were blotted and probed with the 7/1 cDNA clone.

DISCUSSION

During the immunoscreening of an *S. mansoni* λgt11 cDNA library we isolated several clones with mouse immune serum from the 1st to the 7th week of infection. In this paper we have described a 7/1 clone reactive only with 7th week serum, that encodes a fusion protein of 70-kDa. Since the molecular weight of GST is 26 kDa the antigenic determinant is 44kDa. Western blot analysis with mouse immune serum showed that the cDNA clone 7/1 expresses a polypeptide recognized by chronic mouse serum although the corresponding RNA is present in all phases of the parasite during its development in vertebrate hosts.

This antigen named Sm44 has a specific antigenic expression since only serum related to the chronic phase is able to recognize it although in terms of RNA expression the Sm44 antigen of *S. mansoni* is also present in other evolutionary phases of parasitism but is excluded by the immune system perhaps by some block due to host molecule incorporation or different distribution during the development of the parasite in vertebrate hosts, similar to other antigens described previously (Aronstein & Strand 1983, Harn et al. 1985, Davies et al. 1986, Stein & David 1986, Bobek et al. 1986, Grossman et al. 1990, Menrath et al. 1995).

Future studies to confirm this observation and the immunological role of the Sm44 antigen in the immune response to *S. mansoni* are planned.

ACKNOWLEDGMENTS

To Vania Fernandez and Olinda Mara Trevilatto for technical assistance.

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