Evidentiation of Paramyosin (Sm-97) as a Modulating Antigen on Granulomatous Hypersensitivity to *Schistosoma mansoni* Eggs

Cristine Hirsch, Claudia Carvalho-Queiroz, Gloria R Franco, Sergio DJ Pena, Andrew JG Simpson*, Alfredo M Goes/+

Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Caixa Postal 486, 30161-970 Belo Horizonte, MG, Brasil *Instituto Ludwig de Pesquisas sobre o Câncer, 01509-010 São Paulo, SP, Brasil

A Schistosoma mansoni *adult worm anionic fraction (PIII) has previously been shown to protect mice against challenge infection and to reduce pulmonary and hepatic granulomatous hypersensitivity. Serum from PIII-immunized rabbit was used to screen a λgt11 cDNA library from* S. mansoni *adult worm in order to identify antigens capable of modulating granulomatous hypersensitivity. We obtained four clones with 400 (Sm-III.11), 900 (Sm-III.16), 1100 (Sm-III.10) and 1300 (Sm-III.12) bp of length. All clone-specific antibodies were able to recognize most of the PIII components. The sequence analysis showed that these clones presented high homology with* S. mansoni *paramyosin (Sm-97). These findings ascribe a new function to this antigen with an important role in modulation of granulomatous hypersensitivity to* S. mansoni *eggs.*

Key words: Paramyosin - modulation - granuloma - *Schistosoma mansoni*

Schistosomiasis mansoni pathology is ascribed to an inflammatory response towards parasite eggs trapped in host tissues (von Lichtenberg 1987, Boros 1989). The intensity of this response can be modulated by host (Ottensen 1979, Goes et al. 1991) and parasite factors (Ellner et al. 1981, Boulanger et al. 1991). Worms and eggs share the same environment and many adult worm antigens are constitutively shedded in flow blood (Simpson et al. 1984). These antigens can crossreact with egg components (Dunne et al. 1988, Goes et al. 1989) and could be able to modulate the immune response against eggs.

On experimental models, the protective immunity has been studied using a number of antigens isolated from parasite forms (Simpson & Cioli 1987, Butterworth 1992). Many of these parasite components were able to induce moderate levels of protection (Harn et al. 1987, Sher et al. 1986). However, these studies did not analyze the induced granulomatous reactivity to eggs. Besides protection, if granuloma formation could be prevented or suppressed, the development of severe disease might be averted (Boros 1989).

Our laboratory has produced an adult worm anionic fraction (PIII) by separation of adult worm antigen preparation (SWAP) using anionic exchange chromatography on FPLC system (Hirsch & Goes 1996). Immunization of mice with PIII in the presence of *Corynebacterium parvum* and $Al(OH)_3$ as adjuvant induced an immune response that caused a significant protection degree against a challenge infection (Hirsch et al. 1997). In addition, we also showed that PIII induced a significant decrease in granuloma formation to eggs in the liver and lungs of vaccinated mice (Hirsch et al. 1997). Therefore, the interest in the understanding of immunobiology of granuloma response and the role of purified PIII components in the induction of regulatory events that modulate the granulomatous hypersensitivity to *S. mansoni* eggs, has directed our efforts to the characterization of PIII components at molecular level. In this study, we report the identification of *S. mansoni* cDNA clones which encode adult worms antigens recognized by rabbit serum specific for PIII.

MATERIALS AND METHODS

Antisera - Serum was obtained from rabbits immunized by i.m. injections of 100 μg/ml of PIII or SWAP in Complete Freund Adjuvant, boosted

This investigation received financial assistance from Conselho Nacional de Pesquisa (CNPq), Pró-Reitoria de Pesquisa da Universidade Federal de Minas Gerais (PRPq-UFMG) and Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG).
+Corresponding author. Fax:+55-31-441.5963. E-mail: goes@mono.icb.ufmg.br
Received 16 April 1997
Accepted 30 June 1997

twice at two weeks intervals with identical antigen amounts, but in Incomplete Freund Adjuvant.

Antibody select solutions were prepared by plating out phage clone of interest, inducing fusion protein expression by overlaying the plates with isopropyl-β-D-thiogalactopyranoside (IPTG)-soaked nitrocellulose filters, then incubating these filters containing adherent fusion protein overnight in anti-SWAP or anti-PIII rabbit sera. Affinity selected antibodies were then eluted from the filters with 0.1 M glycine buffer pH 2.6, the eluates pooled, neutralized with Tris-HCl, pH 8.0 and fetal calf serum added to 10%.

PIII preparation - PIII was prepared as previously described by Hirsch and Goes (1996). Briefly, SWAP (30 mg) was dialyzed against 20 mM Tris-HCl, pH 9.6, and filtered using an acrodisc 0.2 μm filter. Separation was performed by FPLC (Pharmacia, Upsala, Sweden) on Q-Sepharose anion-exchange chromatography (5 mm x 90 mm glass columns, packed with Q-Sepharose; bead size distribution: 45-165 μm; Pharmacia). Proteins were eluted with 20 mM Tris-HCl, pH 9.6, in a multistep increasing gradient up to 1 M NaCl, interrupted by hold-gradient intervals at 0 (PI), 100 (PII), 280 (PIII), 450 (PIV), 600 (PV) and 750 mM (PVI). Flow-through fractions were concentrated by lyophilization. The concentrated material was dialyzed against 0.15 M phosphate-buffered saline (PBS), pH 7.4, sterilized by filtration and stored at -70°C. The protein content of SWAP fractions was measured according to Bradford microassay (Bradford 1976).

Sodium duodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting - PIII and *S. mansoni* antigen preparations (SWAP) were subjected to discontinuous electrophoresis using SDS-10% polyacrylamide gels, under reducing conditions (Laemmli 1970). The separated proteins were electrophoretically transferred to nitrocellulose paper (Towbin at al. 1979) and then reacted with rabbit sera against SWAP and PIII.

cDNA library screening - An adult worm cDNA library, constructed in the λgt11 vector was screened (Jeffs et al. 1991) using rabbit serum against PIII, the protective anionic fraction from SWAP. Positive phage plaques were selected and re-screened to obtain pure clones. Clone inserts were amplified by Polymerase Chain Reaction (PCR) using λgt11 primers adjacent to the *Eco*RI cloning site (λgt11F: 5'-ggtggcgacgactcctgg agcccg-3' and λgt11R: 5'-ttgacaccagaccaact ggtaatg-3'). The amplification reaction mixture contained 1 μl of the phage suspension in SM buffer, 0.2 μM of each primer, 1.5 mM $MgCl_2$, 200 μM dNTPs and 1U Taq DNA polymerase (Promega) in a 20 μl final volume of a specific reaction buffer (50 mM KCl, 10 mM Tris-HCl pH 9.0 and 0.1% Triton X-100). The thermal profile comprised 30 cycles of denaturation at 96°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 2 min. Amplification products were analyzed on ethidium bromide stained 1% agarose gels.

DNA sequencing and homology searches- Phage inserts from clones Sm-III.10, Sm-III.11, Sm-III.12, Sm-III.16 were PCR amplified using the biotinylated forward primer (λ31F: 5'-gaaggcacatggctgaatatc-3') and the reverse primer (λ183R: 5'-gcgaaatacgggcagaca-3') in a final volume of 100 μl, following the amplification protocol described above. The amplification products were purified using streptavidin coated beads (Dynal). DNA was alkali denatured and the immobilized strand was used for dideoxy chain-termination sequencing with the nested λgt11R primer, essentially as described by Hutman et al. (1989). The sequencing reactions were performed as suggested in the Auto Read Sequencing kit (Pharmacia LKB). The sequencing products were submitted to electrophoresis on a Pharmacia A.L.F. Automated DNA Sequencer. Partial sequences obtained were searched for homology with DNA and protein sequences deposited in databases using the Basic Local Alignment Search Tool (BLAST) (Altshul et al. 1990) e-mail server at the National Center for Biotechnology Information (NCBI) site.

RESULTS

Anti-PIII rabbit serum was able to recognize, by Western blot analysis, components of 160, 97, 45 and 36 kDa after separation on 10% SDS-PAGE under reducing conditions (Figs 1, 2).

This serum was used to screen a λgt11 cDNA library constructed from *S. mansoni* adult worms. Various positive phage plaques were selected and re-screened in order to obtain pure clones (Fig. 3). Four phage preparations were shown to be pure after two re-screenings and all clones contained only one insert after PCR amplification. The sizes of the four inserts were 400 (Sm-III.11), 900 (Sm-III.16), 1100 (Sm-III.10) and 1300 (Sm-III.12) bp long (Fig. 4).

Antibodies specific to the clones were obtained by overnight incubation of filters containing pure phage plaques expressing fusion proteins with anti-PIII polyclonal rabbit serum. Affinity selected antibodies were eluted and used on Western blot assays. These four antibody select solutions recognized most of PIII components separated on reducing conditions in 10% SDS-PAGE (Fig. 5).

The phage inserts were PCR amplified using the biotinylated forward primer (λ31F) and the reverse primer (λ183R) and submitted to solid-phase

sequencing. Partial sequences obtained after single-runs on the A.L.F. DNA sequencer were searched for homology with all DNA and protein sequences deposited in non-redundant databases. All sequences were shown to presented 100% homology with *S. mansoni* paramyosin.

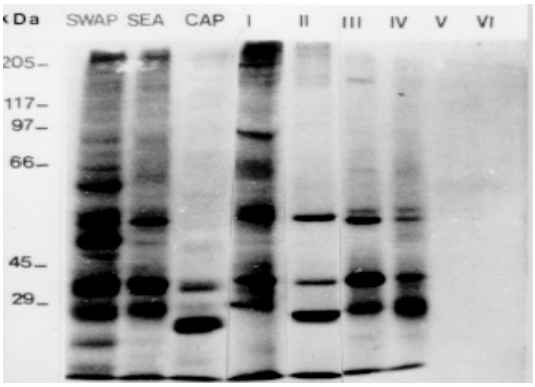

Fig. 1: SDS-PAGE analysis of SWAP fractions from FPLC and other *Schistosoma mansoni* antigen preparations. Aliquots of 20 µg of each antigenic preparation were separated on 10% polyacrylamide gels, under reducing conditions, followed by silver staining. *S. mansoni* antigens: SWAP; SEA; CAP and SWAP fractions - I, II, III, IV, V and VI.

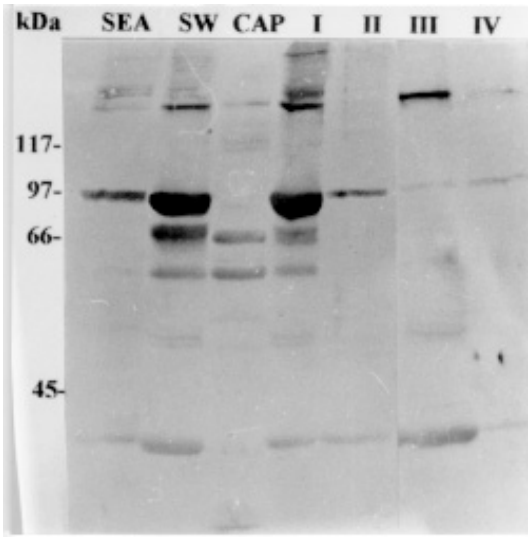

Fig. 2: Western blot analysis of *Schistosoma mansoni* antigens and SWAP fractions, recognized by anti-PIII rabbit serum. Samples prepared from SEA, SWAP (SW), CAP or SWAP fractions (I-IV) were subjected to 10% SDS-PAGE, under reducing conditions, and electrophoretically transferred to nitrocellulose paper. The membrane was incubated with anti-PIII rabbit serum and revealed with goat anti-rabbit IgG conjugated with peroxidase.

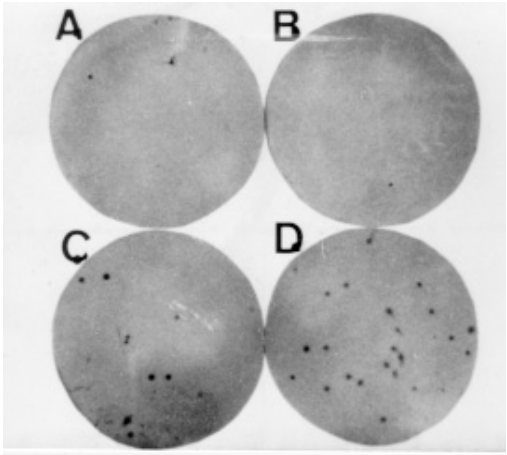

Fig. 3: identification and cloning of *Schistosoma mansoni*-λgt11 recombinants expressing antigenic polypeptides. The plaques were screened with antibodies from rabbit immunized with PIII (A - 1st screen, C-2nd screen, D-clone) or from normal rabbit serum (B). The positive clones were identified using BICP/NBT by incubating with goat anti-rabbit Ig conjugated directly to alkaline phosphatase.

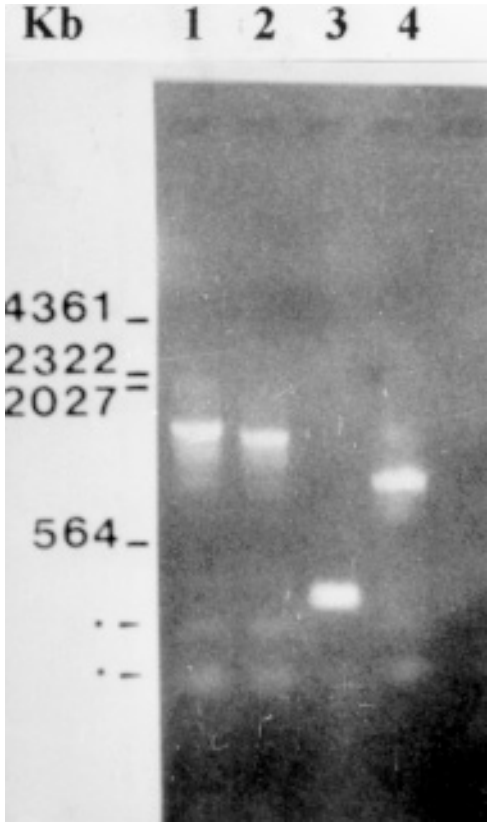

Fig. 4: PCR amplification of *Schistosoma mansoni*-cDNA clones. Ten microlliters of each sample were electrophoresed on 1% agarose containing ethidium bromide (0.5 µg/ml). Lane 1- Sm-III.12 clone; lane 2- Sm-III.10 clone; lane 3- Sm-III.11 clone and lane 4- Sm-III.16 clone.

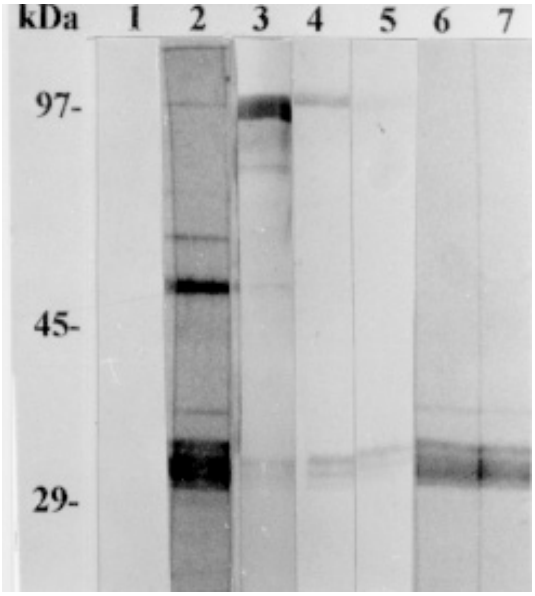

Fig. 5: Western blot analysis of SWAP and PIII probed with antibody selecting solutions against Sm-III.10 and Sm-III.11 clones. SWAP incubated with normal rabbit serum (lane 1); SWAP (lane 2) and PIII (lane 3) incubated with anti-PIII rabbit serum; SWAP (lane 4) and PIII (lane 5) incubated with antibody selected against Sm-III.10 clone; SWAP (lane 6) and PIII (lane 7) incubated with antibody selected against Sm-III.11. Antibodies were probed with a goat alkaline phosphatase-conjugated anti-rabbit Ig.

DISCUSSION

In previous studies on the search of antigens that mediate modulation of granulomatous hypersensitivity against *S. mansoni* eggs, we reported the use of an antigenic protein fraction, obtained from *S. mansoni* adult worm, PIII. This fraction fails to induce vigorous granulomatous hypersensitivity to eggs on liver and lungs, and causes protection against a challenge infection in mice after local (s.c.) immunization. The ability of PIII to suppress both pulmonary and hepatic egg-induced granulomatous responses suggested the possibility of prophylatically immunizing mice against granulomatous inflammation by sensitizing them with adult worm antigens in the presence of *C. parvum* and $Al(OH)_3$. This indicates the presence, within adult worm antigens, of several molecules that might play a role in cell activation and modulation of granulomatous hypersensitivity.

Various experimental approaches have been carried out by investigators to identify target antigens that provide protective immunity and participate as granulomatous modulating agent in schistosomiasis (Harn et al. 1987, Simpson & Cioli 1987, Boulanger et al. 1991, Hirsch & Goes 1996). We believe that molecular cloning and sequencing analysis is an important strategy to identify candidate vaccine antigens against *S. mansoni*.

The data presented here describe four cDNA clones Sm-III.10, Sm-III.11, Sm-III.12 and Sm-III.16 encoding immunologically recognized polypeptides that correspond to proteins contained in PIII, an anionic fraction obtained from SWAP (Hirsch & Goes 1996). The utilization of antibodies specific to the products of these clones was able to identify SWAP proteins with molecular weight of 97 and 35 kDa by western blot analysis. Partial nucleotide sequences of these clones showed 100% of homology with *S. mansoni* paramyosin.

Paramyosin (Sm-97) is the most abundant antigen on *S. mansoni* adult worm. Studies have shown that it is able to induce protection against challenge infection in mice model (Sher et al. 1986). As GST, paramyosin was recognized by reinfection resistant human sera (Correa-Oliveira et al. 1989). Sm-97 was already cloned (Lanar et al. 1986) and its structure is well known (Sher et al. 1986, Lanar et al. 1986, Pearce et al. 1986). Although its capability to induce granulomatous hypersensitivity in footpad swelling assay (Pearce et al. 1986), our studies showed shorter hepatic granuloma reaction induced in immunized mice with PIII (Hirsch & Goes 1996), where paramyosin is a prevalent antigen as we showed in this paper.

In summary, using a powerful rabbit antiserum to screen an adult worm cDNA library, up to 240,000 PFU were analyzed and for four times we obtained clones coding for the muscular protein paramyosin. These findings suggest a prevalence of this antigen in PIII anionic fraction and our results ascribe a role for paramyosin in granuloma modulation.

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