

## **SCHISTOSOMA MANSONI: IDENTIFICATION OF A 46KDa ANTIGEN OF THE SCHISTOSOMULAR SURFACE BY MONOCLONAL ANTIBODY**

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### SUMMARY

An IgG<sub>2a</sub> subclass monoclonal antibody, C<sub>6</sub>G<sub>9</sub>, was obtained by immunization of BALB/c mice with *Schistosoma mansoni* egg antigens. With this monoclonal antibody, it was possible to identify a schistosomular antigen with a molecular weight of 46 kilodaltons (KDa), and its expression being evaluated by means of indirect immunofluorescence. The antigen persisted in the integument of the developing schistosomulum, for at least 96 hours post-transformation. The monoclonal antibody also reacted with the cercaria surface, but not with that of adult worm. The C<sub>6</sub>G<sub>9</sub> was also able to mediate significant levels of cytotoxicity in the presence of complement for newly transformed schistosomula.

**KEYWORDS:** *Schistosoma mansoni*; Monoclonal antibody, Cercariae; Schistosomula.

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### INTRODUCTION

Schistosomula from *Schistosoma mansoni* differ from the adult worms because they are saline-adapted larvae, susceptible to water, derived from cercariae for the loss of the tail, depletion of pre and post-acetabular glands, and with surface changes through significant loss of mucopolysaccharides. In addition to these morpho-physiological transformation, schistosomulum is sensitive to a wide range of "in vitro" immune mechanisms, many of which depend on the identification of surface by antibodies<sup>24</sup>. Thus, it was shown that a complex group of polypeptides, some having antigenic activity, are exposed on the schistosomulum surface<sup>5</sup>.

Once the parasite is established in the mesenteric vessels of human or of many experimental animals, it can stimulate an immunity directed against schistosomulum reinfection without the pre-established parasite population being eliminated. This concomitant im-

munity<sup>23</sup> suggests that surface antigens of the schistosomulum membrane could be the primary target for the immunospecific attack of the host, justifying the great interest in the identification of schistosomulum surface antigens<sup>4, 9, 10, 11, 13, 21, 22, 24, 25, 26</sup>.

In the present work we describe a monoclonal antibody which identifies a 46 KDa component on the schistosomulum surface, that has not been detected on the surface of adult worms.

### MATERIAL AND METHODS

#### *Parasites*

*Schistosoma mansoni* cercariae were obtained from the *Biomphalaria glabrata* kept according to FREITAS et al.<sup>6</sup> at the mollusc nurseery of the Schistosomiasis Research Unit (SRU), at the Institute of Biological Sci-

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ences of Federal University of Minas Gerais, Brazil. Cercariae were mechanically transformed into schistosomula using the method described by RAMALHO-PINTO et al.<sup>14</sup>. Schistosomula of 3, 24, 48, 72 and 96 hours post-transformation were prepared by incubation of newly transformed schistosomula at 37°C in Earle's balanced saline with 0.5% lactoalbumin hydrolysate (ELAC), under a humidified atmosphere and 5% CO<sub>2</sub>. Adult worms were isolated from mice through perfusion of liver and mesenteric vessels, forty-five days after infection. Eggs isolated from the infected livers of mice, after eight weeks infection, were kindly provided by Dr. R. T. Melo. (SRU).

#### *Monoclonal antibody*

BALB/c mice were intraperitoneally immunized with 2000 eggs of *Schistosoma mansoni*, newly isolated from the liver of mice. On the 30<sup>th</sup> day following the initial immunization the animals received intravenous inoculation of 20 µg of soluble egg antigen (SEA). On the 60<sup>th</sup> day, 10 µg of SEA were inoculated intravenously and 200 µg on the 61<sup>th</sup>, 62<sup>nd</sup> and 63<sup>rd</sup> days. Three days after the last dose, spleen cells from the mouse showing the highest strength of anti-SEA antibodies, as detected by immunoenzymatic assay (ELISA), were fused with SP<sub>2</sub>/0 strain myeloma in the presence of polyethylenoglycol (MW 1500), according to GALFRE & MILSTEIN<sup>7</sup>. The cells were dispensed into micro-titre plates containing 100 µl per well of RPMI 1640 plus 15% foetal calf serum (RPMI-FCS) and 10<sup>5</sup> BALB/c mice peritoneal macrophages which had been cultured in the above medium for the previous 24 h. Hybridomas were selected by the addition of hypoxanthine, aminopterin and thymidine. Twenty-one days after fusion each well was tested for antibody by ELISA. Positive wells were selected and cloned by the limiting dilution method in RPMI-FCS and 10<sup>5</sup> BALB/c mice peritoneal macrophages. Large quantities of C<sub>6</sub>G<sub>9</sub> monoclonal antibody were produced by growing hybridomas in BALB/c mice, that were intraperitoneally injected with 0.5 ml of pristine and 7 days later with 10<sup>6</sup> hybridoma cells. Ascitic fluid was collected 2-3 weeks later.

The isotypical class of the C<sub>6</sub>G<sub>9</sub> monoclonal antibody in culture supernatant was determined by agar-radial gel diffusion, according to OUCHTERLONY<sup>12</sup>, using rabbit anti-mouse IgM, IgG<sub>1</sub>, IgG<sub>2a</sub> and IgG<sub>2b</sub> immunoglobulins.

#### *Mouse sera*

Immune sera were collected from mice between 13

to 16 weeks following primary chronic infection with about 50 viable cercariae.

#### *Immunoenzymatic assay (ELISA)*

The ELISA was standardized in a 96 wells polystyrene chloride microtechnique plates (HEMOBAG - Brasil). The adopted antigens were SEA, total extracts from cercariae, schistosomula and adult worms, as well as a preparation of schistosomulum integument obtained by the extraction with calcium chloride, as described by BENNETT & SEED<sup>2</sup>. All antigenic preparations contained protease inhibitors (PMSF, TLCK and TPCK) in the final 2mM concentration. The antigens were used in a final protein concentration of 10 µg/ml as determined by the BRADFORD's method<sup>3</sup>. The monoclonal antibody ascitic fluid and the normal and immune sera were used in a 1:100 dilution.

#### *Indirect Immunofluorescence*

The indirect immunofluorescence assay was used to evaluate the persistence of the 46KDa antigen in the integument during the development of the parasite. Live cercariae and schistosomula of 3, 24, 48, 72 and 96 hours after the mechanical transformation were used.

The adult worms (males and females) were included in cryoform (Cryoform-USA) and superfine sections (10 µm) cut in a cryostat at -20°C. The slides were fixed in 0.7% cold acetone and kept at -20°C until used. Parasite sections as well as cercariae and schistosomula were incubated with a 1:100 dilution of the ascitic fluid in 0.1M pH 7.2 phosphate-buffer for 30 min. at room temperature and washed three times in phosphate buffer. Cercariae, schistosomula and the adult worms sections were incubated again for 30 min. at 4°C with a 1:20 dilution of rabbit anti-mouse IgG labeled with fluorescein isothiocyanate, according to GODDING<sup>8</sup>. After 3 washes in phosphate-buffer, the preparations were examined under a fluorescence microscope. The surface fluorescence intensity was visually scored based on an arbitrary scale, ranging from one to three crosses. Positive and negative controls were included replacing the monoclonal antibody for mouse immune serum and normal mouse serum. In each step, cercariae and schistosomula were immobilized by early exposure to ice bath.

#### *"In vitro" Cytotoxicity in the presence of complement*

This experiment was conducted in order to investigate the susceptibility of schistosomulum to the com-

bined action of the monoclonal antibody and complement, as described by TAVARES et al. <sup>24</sup>, with some modifications.

Twenty-five microliters aliquots of ELAC medium containing 200 newly transformed schistosomula were distributed in hemolysis tubes (75 x 10 mm). Fifty microliters of the C<sub>6</sub>G<sub>9</sub> monoclonal antibody were added, as well as sera from normal and chronically infected mice used as controls. Mice sera and ascitic fluid were previously inactivated. Tubes were kept at room temperature for 30 min, with occasional agitation, and then washed twice in 450 µl of ELAC. Next, 50 µl aliquots from each tube were distributed in excavated plates to count the dead schistosomula. The tests were done in duplicates and repeated in two different occasions to assure the reproducibility of results.

#### Immunoprecipitation

Immunoprecipitation was used to characterize the antigen recognized by the C<sub>6</sub>G<sub>9</sub> monoclonal antibody. This stage was performed according to the method proposed by SIMPSON et al. <sup>18</sup>. In brief, artificially transformed schistosomula were surface labeled with I<sup>125</sup> using Iodogen method. Labeled polypeptides were solubilized in 1% Triton X-100 in 150 mM NaCl, 50 mM Tris-HCl, 50 mM EDTA, pH 8.0 containing 1mM PMSF. Antigens were then identified by immunoprecipitation, utilizing Protein A-Sepharose and electrophoresis in SDS-poliacrylamide gel.

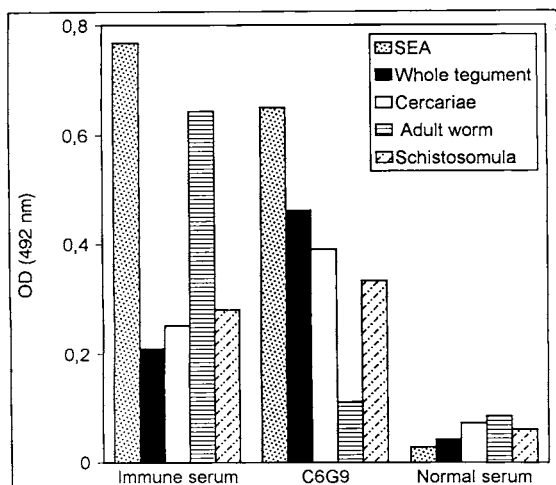


Figure 1 - Reactivity of the monoclonal antibody (C<sub>6</sub>G<sub>9</sub>) against different antigenic preparations of *Schistosoma mansoni*, by immunoenzymatic assay (ELISA).

## RESULTS

#### Class and specificity of C<sub>6</sub>G<sub>9</sub> monoclonal antibody

The fusion carried out between the spleen cells of the immunized mouse with *Schistosoma mansoni* egg antigen (SEA) and the SP<sub>2</sub>/0 myeloma provided several hybridomas. After cloning by limiting dilution, 20 clones were isolated. One of these, designated C<sub>6</sub>G<sub>9</sub>, showed IgG<sub>2a</sub> monoclonal antibody secretion as assayed in radial agar-gel diffusion against anti-mouse immunoglobulins antiserum.

This C<sub>6</sub>G<sub>9</sub> monoclonal antibody presented reactivity with egg, cercariae and schistosomulum antigens in ELISA, but it reacted weakly with adult worm extracts. A higher reactivity seems to occur with schistosomulum integument antigens in relation to schistosomula total extract (Figure 1).

#### Persistence of the antigen identified by C<sub>6</sub>G<sub>9</sub> monoclonal antibody during the development of *Schistosoma mansoni*

This study was carried out to verify how long the monoclonal antibody could still detect the surface antigen after cercaria transformation by indirect immunofluorescence. Table 1 relates presence of C<sub>6</sub>G<sub>9</sub> and each development stage of the parasite. The monoclonal antibody showed a linear and continuous surface fluorescence, for the whole cercaria and schistosomulum body, up to 96 hours of "in vitro" culture. Concerning the adult worms, C<sub>6</sub>G<sub>9</sub> has not been able to interact with any antigen of this developmental stage.

#### "In vitro" Cytotoxicity of C<sub>6</sub>G<sub>9</sub> monoclonal antibody

Data obtained from this experiment are shown in Figure 2. The monoclonal antibody, in the presence of complement, has mediated cytotoxicity levels for the schistosomula in lower proportions than the chronically

TABLE 1

Persistence of the antigen detected by C<sub>6</sub>G<sub>9</sub> monoclonal antibody in the surface of different development stages of *Schistosoma mansoni* through immunofluorescence test.

Evolutionary stage of <i>S. mansoni</i>	Immune serum	C <sub>6</sub> G <sub>9</sub>	Normal serum
Cercaria	++	++	-
3 h schistosomulum	++	++	-
24 h schistosomulum	+++	++	-
48 h schistosomulum	+++	+	-
72 h schistosomulum	+++	+	-
96 h schistosomulum	+++	+	-
Adult worm	+++	-	-

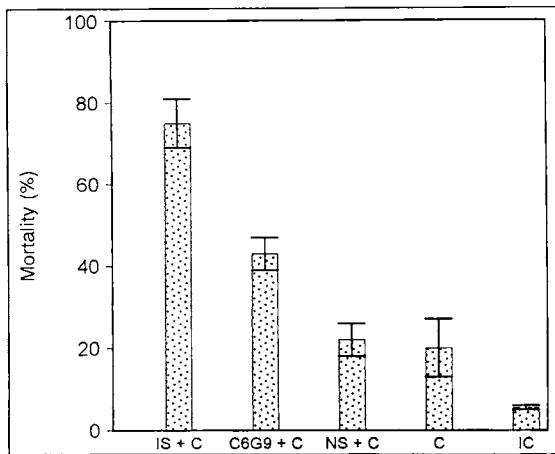


Figure 2 - Comparative susceptibility of schistosomula to combined action of C<sub>6</sub>G<sub>9</sub> monoclonal antibody and complement. IS - immune serum; C - complement; NS - normal serum; IC - inactivated complement.

infected mice immune serum, although significantly higher (p<0.01) as compared to the action of normal serum.

Invariably, in this kind of experiment, schistosomula incubation with only the complement presented approximately 15 to 20% mortality. The cytotoxic strength of the complement was practically abolished, when male guinea-pig serum complement was inactivated, showing clearly that no other component from the culture medium could be toxic to the parasites.

**Identification of the surface antigen recognized by C<sub>6</sub>G<sub>9</sub> monoclonal antibody**

Immunoprecipitation of schistosomulum surface proteins by C<sub>6</sub>G<sub>9</sub> monoclonal antibody originated an autoradiograph in which a single band with apparent molecular weight of 46 KDa was identified (Figure 3 - track 3). The monoclonal antibody reacted specifically with this surface component, since the mouse normal serum has not immunoprecipitated any antigen (Figure 3 - track 2).

**DISCUSSION**

Monoclonal antibodies have many applications in the schistosomiasis study. It is important to have antibody secreting clones able to identify the main surface antigens of schistosomulum, which is known to be the most vulnerable stage to the host immune response.

In this study, it was described a monoclonal anti-

body produced against a soluble antigen of the *Schistosoma mansoni* egg, with specificity for a schistosomulum surface antigen, as it was observed in the indirect immunofluorescence reaction and ELISA, using schistosomulum integument antigens. Our immunoprecipitation showed that this antigen which remains on the schistosomulum surface has a molecular weight of about 46 KDa. It has not been detected in adult worms, and it is able to mediate a significant "in vitro" cytotoxicity effect to transformed schistosomula in the presence of complement.

SIMPSON<sup>17</sup> classified antigens associated with the schistosomulum surface into two categories, based on their expression during the parasite life cycle: those sharing the parasite egg and other *Schistosoma* species; and those sharing the adult worm, but not the egg. According to this standpoint, the antigen detected by the studied monoclonal antibody fits the first category, because this monoclonal antibody was obtained from mouse immunized with antigen from the parasite egg, and showed itself reactive with schistosomulum, but not with adult worm.

It must be pointed out that the 46 KDa antigen described in this work is not possibly related to a 45 KDa

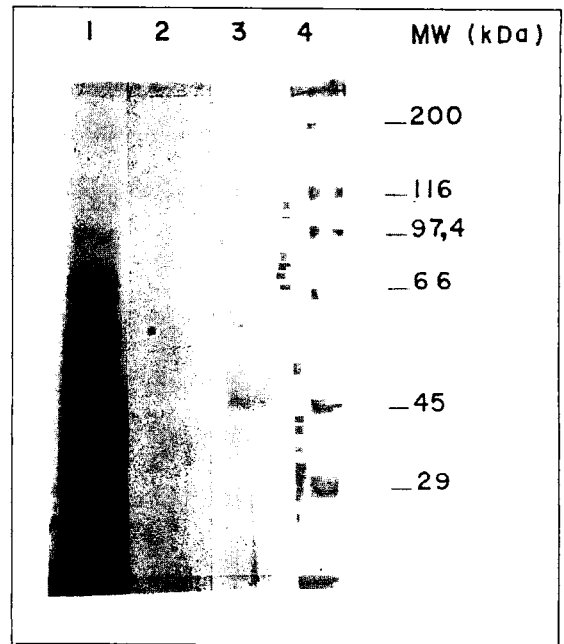


Figure 3 - Immunoprecipitation of schistosomulum surface proteins by C<sub>6</sub>G<sub>9</sub>. Track 1 - Whole Tegument Radiolabeled; Track 2 - Normal Serum; Track 3 - C<sub>6</sub>G<sub>9</sub>; Track 4 - Standards of molecular weight.

protein extracted and identified by RUMJANECK et al.<sup>16</sup>, since this 45 KDa lipoprotein receptor is only expressed on the schistosomulum surface after incubation with human serum. In addition, this molecule presented itself as a double band in the polyacrylamide gel. This fact was not evidenced in the autoradiograph of the studied 46 KDa antigen.

One of the schistosomulum surface polypeptides, clearly defined in terms of molecular homogeneity, is a 38 KDa component identified by DISSOUS et al.<sup>5</sup> using serum from infected animals. This important antigen is possibly different from the surface component described in the present study, because according to SIMPSON et al.<sup>19</sup>, the 38 KDa peptide disappears from the parasite surface during the first 48 hours of "in vitro" culture.

In the same way, CHEN et al.<sup>4</sup> and SMITH et al.<sup>20</sup> reported that a 50 KDa antigen, in addition to being detected in eggs, cercariae and schistosomulum, has also been observed in adult females, thus suggesting it is another antigenic component.

On the other hand, ROBERTS et al.<sup>15</sup>, when labeling adult worms by lactoperoxidase method, identified thirteen protein bands including one with 44-46 KDa.

Also it was found on the surface of schistosomula mechanically transformed, opposing the features of the 46 KDa found in the present work. It must be pointed out, however, that the used surface protein labeling method in our experiments (Iodogen) differs from the one employed by those investigators.

Efforts will be made in order to achieve the isolation of this antigen for immunization studies in isogenic mice, so as to observe whether this studied schistosomular surface component would be able to mediate a protective effect against the cercariae challenge.

## RESUMO

### ***Schistosoma mansoni*: identificação de um antígeno de 46KDa da superfície de esquistossômulo por anticorpo monoclonal.**

Um anticorpo monoclonal da subclasse IgG<sub>2a</sub>, designado C<sub>6</sub>G<sub>9</sub>, foi obtido pela imunização de camundongos BALB/c com antígenos de ovo de *Schistosoma mansoni*. Esse anticorpo monoclonal possibilitou a identificação de um antígeno de peso molecular aproximado

de 46 quilodaltons (KDa), cuja expressão foi avaliada através da reação de imunofluorescência indireta. O referido antígeno persistiu no tegumento do esquistossômulo em desenvolvimento pelo menos até 96 horas pós-transformação. O anticorpo monoclonal reagiu também com a superfície de cercárias, mas não com a de vermes adultos. O C<sub>6</sub>G<sub>9</sub>, em presença de complemento, foi também capaz de mediar níveis significativos de citotoxicidade para esquistossômulos recém-transformados.

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## REFERENCES

1. ARONSTEIN, W. S.; NORDEN, A. P. & STRAND, M. - Tegumental expression in larval and adult stages of a major schistosome structural glycoprotein. *Amer. J. trop. Med. Hyg.*, 32: 334-342, 1983.
2. BENNETT, L. J. & SEED, J. L. - Characterization and isolation of Concanavalin A binding sites from the epidermis of *Schistosoma mansoni*. *J. Parasit.*, 63: 250-258, 1977.
3. BRADFORD, M. M. - A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem.*, 72: 248-254, 1976.
4. CHEN, L.; REKOSHI, D. M. & LOVERDE, P. T. - *Schistosoma mansoni* p48 eggshell protein gene: characterization, developmentally regulated expression and comparison to the p14 eggshell protein gene. *Molec. Biochem. Parasit.*, 52:39-52, 1992.
5. DISSOUS, C.; GRZYCH, J. M. & CAPRON, A. - *Schistosoma mansoni* surface antigen defined by a rat monoclonal IgG<sub>2a</sub>. *J. Immunol.*, 129: 2232-2234, 1982.
6. FREITAS, J. R.; RESENDE, E. S.; JUNQUEIRA, D. V.; COSTA, A. M. & PELLEGRINO, J. - Criação em massa e ritmo de crescimento da *Biomphalaria glabrata*. *Cienc. e Cult.*, 23:968-974, 1975.
7. GALFRE, G. & MILSTEIN, C. - Preparation of monoclonal antibodies: strategies and procedures. In: SYMPOSIUM OF THE PROPERTIES OF THE MONOCLONAL ANTIBODIES PRODUCED BY HYBRIDOMA TECHNOLOGY AND THEIR APPLICATION TO THE STUDY OF DISEASES, Singapore, 1981. Geneva, UNDP/WORLD BANK/WHO, 1982. *Proceedings*. p. 1-46.
8. GODDING, J. W. - Conjugation of antibodies with fluorochromes: modifications to the standard methods. *J. Immunol. Meth.*, 13: 215-226, 1976.
9. HARN, D.; MITSUYAMA, M. & DAVID, J. R. - *Schistosoma mansoni*: anti-egg monoclonal antibodies protect against cercarial challenge *in vivo*. *J. exp. Med.*, 159: 1371-1387, 1988.
10. HAYUNGA, E. G.; MURRELL, K. D.; TAYLOR, D. W. & VANNIER, W. E. - Isolation and characterization of surface antigens

- from *Schistosoma mansoni*. II. Antigenicity of radiolabelled proteins from adult worms. *J. Parasit.*, 65: 497-506, 1979.
11. OMER-ALI, P.; HAGAN, F.; HACKETT, F.; SMITHERS, S. R. & SIMPSON, A. J. G. - Variable species and stage specificity of schistosomulum surface epitopes recognized by mice vaccinated with highly irradiated cercariae. *Paras. Immunol.*, 11: 257-267, 1989.
  12. OUCHTERLONY, O. & NILSSON, L. A. - Immunodiffusion and immunoelectrophoresis. In: WEIR, D. M. - *Handbook of experimental immunology*. 2 ed. Oxford, Blackwell, 1973. v. 1, p. 19. 1-19.39.
  13. PAYARES, G.; McLAREN, D. J.; EVANS, W. H. & SMITHERS, S. R. - Changes in the surface antigen profile of *Schistosoma mansoni* during maturation from cercariae to adult worm. *Parasitology*, 91: 83-99, 1985.
  14. RAMALHO-PINTO, F. J.; GAZZINELLI, G.; HOWELLS, R. E. et al. - *Schistosoma mansoni*: defined system for step-wise transformation of cercariae to schistosomula *in vitro*. *Exp. Parasit.*, 36: 360-372, 1974.
  15. ROBERTS, S. M.; AITKEN, R.; VOJVODIC, M.; WELLS, E. & WILSON, R. A. - Identification of exposed components on the surface of adult *Schistosoma mansoni* by lactoperoxidase-catalysed iodination. *Molec. Biochem. Parasit.*, 9: 129-143, 1983.
  16. RUMJANECK, F. D.; McLAREN, D. J. & SMITHERS, S. R. - Serum induced expression of a surface proteins in schistosomula of *Schistosoma mansoni*: a possible receptor for a lipid uptake. *Molec. Biochem. Parasit.*, 9: 337-349, 1983.
  17. SIMPSON, A. J. G. - Schistosome surface antigens: developmental expression and immunological function. *Parasit. today*, 6: 40-45, 1990.
  18. SIMPSON, A. J. G.; JAMES, S. L. & SHER, A. - Identification of surface antigens of schistosomula of *Schistosoma mansoni* recognized by chronic infection and by exposure to highly irradiated cercariae. *Infect. Immun.*, 41: 591-597, 1983.
  19. SIMPSON, A. J. G.; PAYARES, G.; WALKER, T.; KNIGHT, M. & SMITHERS, S. R. - The modulation of expression of polypeptide surface antigens on developing schistosomula of *Schistosoma mansoni*. *J. Immunol.*, 133: 2725-2730, 1984.
  20. SMITH, A. L., HUGGINS, M. C. & HAVERCROFT, C. - *Schistosoma mansoni*: adult males and females differentially express antigens encoded by repetitive genomic DNA. *Parasitology*, 105: 63-69, 1992.
  21. SMITH, M. A. & CLEGG, J. A. - Vaccination against *Schistosoma mansoni* with purified surface antigens. *Science*, 227: 535-538, 1985.
  22. SMITH, M. A.; CLEGG, J. A. & SNARY, D. - Monoclonal antibodies demonstrate similarity of surface antigens on different clones of *Schistosoma mansoni* schistosomula. *Trans. roy. Soc. trop. Med. Hyg.*, 78: 187-189, 1984.
  23. SMITHERS, S. R.; TERRY, R. J. & HOCKLEY, D. J. - Host antigens in schistosomiasis. *Proc. roy. Soc. B.*, 171: 483-494, 1969.
  24. TAVARES, C. A. P.; DE ROSSI, R.; PAYARES, G. et al. - A monoclonal antibody raised against adult *Schistosoma mansoni* which recognizes a surface antigen on schistosomula. *Z. Parasitenk.*, 70: 189-197, 1984.
  25. TAYLOR, D. W. & BUTTERWORTH, A. E. - Monoclonal antibodies against surface antigens of schistosomula of *Schistosoma mansoni*. *Parasitology*, 84: 65-82, 1982.
  26. ZODDA, D. M. & PHILLIPS, S. M. - Monoclonal antibody mediated protection against *Schistosoma mansoni* infection in mice. *J. Immunol.*, 129: 2326-2328, 1982.
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