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RESEARCH ARTICLE

Evaluation of immunization with purine salvation pathway recombinant enzymes in *Schistosoma mansoni* worms and eggs in murine schistosomiasis

Débora Meira Neris[1], Humberto D'Muniz Pereira[2], Ricardo de Oliveira Correia[1], Sandra Regina Pereira de Oliveira[1], Naiara Naiana Dejani[1], Joice Margareth de Almeida Rodolpho[1], Laís Cristina de Souza[1], Ana Carolina Maragno Fattori[1], Larissa Romanello[2], Juliana Roberta Torini de Souza[2], Vanderlei Rodrigues[3], Fernanda de Freitas Anibal[1]

1. Laboratory of Parasitology, Department of Morphology and Pathology, Federal University of São Carlos-UFSCar- São Carlos (SP), Brazil .
2. Center for Structural Molecular Biotechnology, São Carlos Institute of Physics, University of São Paulo –USP – São Carlos (SP), Brazil.
3. Ribeirão Preto School of Medicine - University of São Paulo – USP – Ribeirão Preto (SP), Brazil.

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Abstract

According to the World Health Organization (WHO), on tropical and subtropical areas, schistosomiasis is the second parasitic disease of greater prevalence, in terms of morbidity and mortality, surpassed only by malaria. The Praziquantel (PZQ) is used for the treatment of this disease. However, reports of resistant strains reinforce the need to develop a new schistosomicidal drug. The infection by the parasite induces an inflammatory reaction of long duration due to the presence of adult worms living in the mesenteric venous system. The parasite lays eggs in small vessels of the submucosa of the intestines. These eggs are transported by the blood flow to the liver and they cause a granulomatous inflammatory reaction. A new approach can be held by the study of the following *Schistosoma mansoni* enzymes: purine nucleoside phosphorilase 1 (PNP), hypoxanthine guanine phosphoribosyltransferase (HGPRT) and adenylate kinase (ADK). The parasite, incapable of synthetizing purine nucleotides through the *de novo* pathway, has multiple mechanisms to incorporate purine bases through the purine salvage pathway. In our results, we suggest that the immunization in Balb/c mice with the mentioned recombinant enzymes was capable of inducing a specific immune response, favoring the reduction of both the parasite load and number of eggs per gram of feces. The acquired data show that these enzymes can be considered as new targets to immunotherapy against schistosomiasis mansoni.

1. Introduction

According to the World Health Organization (WHO), schistosomiasis is the second most predominant parasitic disease in tropical and subtropical areas; it is only surpassed by malaria when we compare the morbidity and mortality rates of these two diseases. About 779 million people of 77 endemic countries live in the areas where the risk of contamination is high (WHO, 2012). This disease is caused by a trematode of the *Schistosoma* genus which affects mainly the populations in developing countries (Hotez & Ferriz, 2006). The World Health Organization recommends the use of Praziquantel (PZQ) to treat the schistosomiasis; however, this medicine leads to some mutagenic and teratogenic side effects, and in the last 30 years, no new drugs have been developed to deal with this disease (Novaes et al., 1999; Shu-hua, 2005). The *S. mansoni* is a complex organism; it has specific antigens in each

phase of its development: cercariae, schistosomula, adult worms and eggs. The infection by the parasite induces an inflammatory reaction of long duration due to the presence of adult worms living in the mesenteric venous system. The parasite lays eggs in small vessels of the submucosa of the intestines. These eggs are transported by the blood flow to the liver and they cause a granulomatous inflammatory reaction (Oliveira et al., 2011). The tegument of the parasite contains some proteins that act as a barrier and, consequently, create a protection against the attack of the host's immune system. The tegument has also the capacity of incorporating certain proteins found in the host trough a passive mechanism; thus, the parasite can be unobserved in the host's immune system (Braschi & Wilson, 2006). About 740 proteins of the *S. mansoni* were identified, and they could be classified in three categories: (1) the tegument only, (2) the body of the parasite only, and (3) both the tegument and the body of the parasite. A total of 222 proteins were found in the tegument (Braschi et al., 2006). The discovery of these specific proteins is of great interest for the scientific community because they are the first ones to be presented to the host's immune system. For this reason, these proteins provide substantial elements for future studies that are centered on the development of vaccines (Verjovski-Almeida et al., 2003). The acute phase of the disease is quite debilitating for human beings. They present fever even before the eggs appear in the feces (Rabello, 1995). Plasma levels of the tumor necrosis factor (TNF) also occur. These levels increase because of the peripheral blood mononuclear cells (PBMCs). The production of cytokines by the PBMCs, after stimulation with the parasite's antigens, reflects on a Th1 response. However, as the disease progresses, the antigens of the eggs induce aTh2 response (Montenegro et al., 1999). The most serious form of chronic schistosomiasis is caused by the hepatosplenomegaly, which is generally accompanied by severe hepatic fibrosis, periportal and portal hypertension (Dunne & Pearce, 1999). In the infected host, the disease is characterized by the presence of granuloma. The granuloma is formed by: several inflammatory cells of the immune system, components of the extracellular matrix, adhesion proteins, growth and angiogenesis factors. All of these elements are in the origin of a spherical structure that envelops each egg individually. After the death of the egg, the granuloma decreases and leaves in its place fibrous plaques that increase the portal blood pressure and the diameter of the portal vein (Lenzi et al., 2008; Pearce & Macdonald, 2002). This reaction shows the importance of the egg as a pathogenic agent; about half of the eggs produced by the worms reach the intestinal light and are eliminated by the feces (Rey, 2008). As a result of several granulomatous reactions in the liver, the functions of this organ are altered (Jankovic et al., 1997); therefore, such reactions can cause severe hepatic failures. The *S. mansoni* causes a low natural immunity and they are able to evade or resist to the specific immune responses of the hosts. The propagation of the disease is even more pronounced by the lack of infrastructure of water resources, deficiency in people's education concerning the domestic and hygienic habits and absence of sanitary education (Filho & Silveira, 2001). Given this, the study of the enzymes that participate in the *S. mansoni* essential pathways can be quite promising to develop specific tools to control this illness. The nucleotides of purines are required in all living beings to DNA and RNA synthesis. These nucleotides can be acquired by the *de novo* synthesis or by the salvage pathway of the purines. The *de novo* pathway uses simple precursors to the synthesis of several nucleotides. On the other hand, the salvage pathway of purines is the reutilization of pathways by which the organism can satisfy its requirement of pre-formed purines (el Kouni et al., 1987). One of the advantages of the salvage pathway is the fact that the *de novo* pathway demands a big quantity of energy to the purine bases (Voet & Voet, 1995). Different from its human host, the *S. mansoni* depends entirely on the salvage pathway of purines to feed its demand of purine bases (Senft et al., 1972). Another relevant factor to this research is the fact that the literature does not have sufficient data to respond to the questions about the mechanisms of action of these enzymes. Such enzymes act in an intracellular manner, and no scientific works which can prove their exposition or secretion by the *S. masoni* have been found. Therefore, essential enzymes to the parasite can be promising study subjects. The enzymes hypoxanthine guanine phosphoribosyltransferase (HGPRT) (E.C 2.4.2.8), adenylate kinase (ADK) (E.C 2.7.4.3) and purine nucleoside phosphorilase (PNP) (EC 2.4.2.1), are cytoplasmic enzymes and its exposure in the adult worm's tegument were not yet verified. Some studies which reports secretion of one of the enzymes component of the *S. mansoni* purine salvation pathway, the nucleoside-diphosphate kinase (NDPK), by prokaryote pathogens, as showed by Zaborina (1999) in which was observed secretion of this enzyme by *Mycobacterium bovis* e *Pseudomonas aeruginosa* bacteria, as well as Punj (2000). Recently a group of collaborators with this work (IFSC-USP) showed that the structures of the PNP1, HGPRT and ADK enzymes do not present any danger as for the immunization in human beings: the highest percentages of homology were between 30-50% of identity with the same in human beings. However, in order to occur an immunological response, it is fundamental to have some accessible factors (antigens exposed) and these external antigenic factors are the first to be recognized; with the time being, with the disruption of some of the antigens, more internal factors can be expressed or excrete physiologically. The innate immune system is able to identify antigenic parts present in the pathogen surfaces known as PAMPs (Pathogen-Associated Molecular Pattern*)* and then the first patterns of immunological response against the pathogen is initiated. The pathogenesis of the schistosomiasis mansoni is dependent on the human interaction with the helminth,

being important factors: the strain, the evolutive phase, the intensity and the number of infections. The acute phase is divided in pré-patent before the oviposition and the post-patent after the oviposition. Even though the immunological mechanisms involved in the response of the acute infection are not yet completely explained, some investigations in murine models indicated that there is in the beginning a predominance of an immune response type Th1, substituted by Th2 in the post-patent phase (Silva et al., 2008). In the pre-patent phase, plasmatic levels considered of tumor necrosis (TNF) and of Interleukins 2 and 6 produced by the mononuclear cells (Pearce & Macdonald, 2002). It is supposed that the immune response type Th1 is responsible for the damages in the tissues and the clinic manifestations in the acute phase. Another interesting factor is the cellular cytotoxicity dependent on the antibodies (ADCC), with effector action on schistosomula. Lately the Oswaldo Cruz Institute (IOC), located in Rio de Janeiro, Brazil, produced and patented the first vaccine to the schistosomiasis in the world. The vaccine, approved by the National Agency of Sanitary Supervision (ANVISA/Brazil), demonstrated to be safe and able to immunize human beings against the disease. The national dose was produced from an antigen Sm14, being part of the fatty acid-binding proteins (Tendler et al., 1995). In 2008, the RNAi technique reinforced the importance of the enzyme HGPRT to the survival of the parasite. Small RNAs of interference (RNAis) were produced against enzyme, injected after 70 days of infection with cercariae in mice. The number of worms was counted six days after the injection (Pereira et al., 2008). The work by Peng (2004) that demonstrated in serums of rabbits infected with cercarie *Schistosoma japonicum* a positive immune response with the enzyme adenylate kinase. Two kinds of pathways lead to nucleotide synthesis in the living beings: *de novo* and salvation pathways. The de novo pathways for purine and pyrimidine biosynthesis are similar in almost all living organism and have as metabolic precursors: amino acids, ribose 5- phosphate, CO2 e NH3 (Freeman, 2008). The salvation pathways are the reutilization of nucleosides that were released from the break of nucleic acids. Thus, one of the advantages of the salvation pathway is that it requires less amount of energy for purine bases synthesis, compared to the *de novo* pathway. In parasite have demonstrated a fast adenine incorporation in nucleotides and were not able to establish 14C-glycine and 14C-glucose incorporation in the purine ring (Senft et al., 1972). This shows great dependence of an external supply of pre-formed bases for nucleotide synthesis, as shown by the loss of the purine synthesis of the de novo pathway. The HGPRT enzyme catalyzes the reversible phosphorylation from hypoxanthine and guanine to inosine monophosphate or guanosine monophosphate, respectively, and pyrophosphate using as phosphate and ribose donor the 5-phosphoribosyl- diphosphate (PRPP). Researches on *S. mansoni* schistosomula indicates that organisms basically depends on the purine bases retrieval in order to ensure its purine nucleotide needs (Dovey et al., 1986). Due to limited interconversion between adenine and guanine nucleotides, the *S. mansoni* HGPRTase provides the main sources of guanine nucleotides for the parasite. The enzyme has been found usually as a dimer in solution on *Tritrichomonas foetus* (HGXPRT) (Somoza et al., 1996). *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania tarentolae* (Monzani et al., 2002) and *Leishmania donovani* (Ullman et al., 1997) organisms. It is found in the tetramer form in solution on *Toxoplasma gondii* (HGXPRT) (Héroux et al., 1999), *Escherichia coli* (HPRT) (Gudatt et al., 2002). *Plasmodium falciparum* (HGXPRT) (Keough et al., 1999) and on human enzyme (Xu et al., 1997). This enzyme has 48% of identity, when compared to the human homolog and demonstrated several properties that distinguishes it from mammal's enzymes (Dovey et al., 1986). Three homologous genes for the HGPRT (Smp_148820, Smp_168500, Smp_103560) were found at the parasite's genome, which are related with the parasite's different life stages. The PNP is an enzyme that reversibly catalyzes the phosphorylase purine nucleoside in order to generate the purine base and ribose-1- phosphate and it has been descripted as participant in the *S. mansoni* salvation purine pathway with the sole purpose of supplying purine bases used in the DNA and RNA synthesis (Senft et al., 1972; Senft et al., 1983). It is important to notice that, in *Schistosoma* adult worms (Senft et al., 1983) and schistosomula (Dovey et al., 1984) unlike its mammal hosts, the parasite are not able to synthesize purine nucleosides and depend solely on the salvation pathway to its purine necessities. Two isoforms were identified in the parasite's genome, named SmPNP1 e SmPNP2. The SmPNP1was the first salvation pathway enzyme which was cloned, expressed, purified, crystallized and had its structure resolved (Pereira et al., 2003; Pereira et al., 2005). The SmPNP1 has 287 amminoacids, being a trimer in solution and in crystal form. The SmPNP2, which has 61,8% of identity, when compared to SmPNP1 and coded to a protein with 299 amminoacids, being greater than SmPNP1. Adenylate kinase (ADK) is an enzyme that is involved in cellular homeostasis, energetic metabolism and nucleotide synthesis. The enzyme catalyzes the ATP (Adenosine triphosphate) + AMP Adenosine monophosphate *ƒ´* 2 ADP (Adenosine diphosphate) reaction. It is known that several ADK are found in mammals, as the skeletal muscle is particularly rich in ADK1, main provider of isoforme citosolica (Tanabe et al., 1993). The ADK1 (Smp_071390) codes to a protein with 197 amminoacids and has 54% of identity, when compared to the human ADK1. The work of Peng (2004) has shown that immunization with the *Schistosoma japonicum* ADK enzyme induced the antibodies production against *S. japonicum*, revealing a positive immune response post-immunization. Thus, our study aimed to assess whether purine salvation pathway enzymes (PNP 1,

HGPRT and ADK), when used as immunizing, associated to the aluminum hydroxide adjuvant, stimulate a specific immune response which favors the *S. mansoni* antigens recognition. Hence, our work intends to assess whether *S. mansoni* PNP1, HGPRT and ADK enzymes are able, futurely, to be candidates for a vaccine against this disease, since it has been demonstraded that these enzymes were able to produce an immunologic response after immunization in balb/c mice (Neris et al., 2013). Thereby, we suggest that these enzymes, by mechanisms yet unknown, seem to modulate the infection by *Schistosoma* in different species of rodents. These facts support the hypothesis that the proposed enzymes, although related to the DNA of the parasite, can be expressed in the *S. mansoni* membrane and excreted by some pathway, which can cause a specific activation during the schistosomiasis mansoni.

2. Material and Methods

2.1 The mice

We used in this experiment female mice of Balb/c lineage and specific pathogen free (SPF). They weighed between 15 and 18 grams, and they were brought from the CEMIB of the University of Campinas (UNICAMP), Brazil. The mice were kept in proper cages, with free access to potable water and standard commercial diet, in the vivarium of the Department of Morphology and Pathology of the University of São Carlos, Brazil. The procedures of this experiment were based upon the Ethical Principles of the Animal Experimentation, adopted by the Brazilian Society of Laboratory Animal Science (SBCAL), and they were submitted and approved by the Ethics Commission in Animal Experimentation (CEEA) of the University of São Carlos.

2.2 Obtaining the *Schistosoma mansoni* cercariae and the infection of the mice

The larvae (cercariae) of *S. mansoni*, strain LE (BH-MG), were gently given in by the Professor Vanderlei Rodrigues, member of the Department of Biochemistry and Immunology of the University of São Paulo-(FMRP/USP). This lineage is routinely maintained by passage through the *Biomphalaria glabrata* snails and the Swiss mice. Infected *B. glabrata* snails were induced to eliminate the cercariae by exposition to artificial elimination 30º C in non-chlorine water bath during 1 hour. The mice were infected using subcutaneous route (s.c.) with 50 cercariae of *S. mansoni* in 0,3 mL of physiological solution 0,9% NaCl/animal. This volume and this concentration (physiological solution) are unable to affect the viability of the cercariae. The method of infection was standardized by the laboratory technician Olinda Mara Brigoto of the Department of Biochemistry and Immunology of the University of São Paulo (FMRP/USP).

2.3 Obtaining of PNP1, HGPRT and ADK recombinant enzymes

The methodology to obtain the PNP1 enzyme was done according to Pereira (2003), and for the HGPRT and ADK enzymes, according to Romanello (2011) The procedure was conducted at the Cristallography Laboratory of the São Carlos Institute of Physics – IFSC, located at University of São Paulo, Brazil – USP, São Carlos – SP – Brazil. The recombinant enzymes were synthesized by the insertion of plasmids in bacterial cultures. To do so, the protein expression methodology was used. The enzymes were purified through the method of affinity chromatography under the coordination of Dr. Humberto D' Muniz Pereira and supervision of Prof. Dr. Richard C. Garratt, both belonging to the same aforementioned institution.

2.4 Immunization of the mice with the recombinant enzymes

After purification, 100µg individually, the recombinant enzymes PNP1, HGPRT and ADK were added to 200µL of sterile PBS, containing 100µg of aluminum hydroxide (dynamics 35044) and injected thrice into the mice through intraperitoneal route (i.p) in intervals of 15 days. After 15 days since last immunization, the mice were infected with 50 cercariae/animal of *S. mansoni*. The experimental groups consisted of a control group of 6 mice, which did not receive any kind of immunization, a group which was infected only, and a group of 6 mice, which was immunized (PNP1 or HGPRT or ADK) and then infected. The model proposed in the experiment was repeated twice in order to confirm the results. The mice were euthanized on the 48^{th} and 85^{th} days after the infection with the Thionembutal anesthetic (North Chicago, Illinois, USA). The days to perform the euthanasia were established on the 48^{th} and 85^{th} days due to the facts that the parasite had already started laying eggs and the disease had already reached its chronic phase, respectively.

2.5 Counting the eggs in the feces

On the 47th and 84th days after the infection, the feces of the mice were collected and examined. For the analysis, we used the Kato-Katz kit (Katz & Peixoto, 2000). This kit, through the Helm Test, is developed by the Immunobiological Technology Institute (Biomanguinhos, Fiocruz – Brazil) and recommended by the World Health Organization because it increases from 2 to 4 times the chances of detecting the eggs of the helminths in the feces. The Kato-Katz method does not need water, light or any other accessory to prepare the blades, which can be conserved in room temperature for up to two years. The procedures for the analysis were executed following the manufacturer's instructions. The feces were submitted to the sieving filter-Test, mounting on microscope slides and covered with cellophane cover slip pre-colored with malachite green pigment. After counting the eggs, we calculated the quantity of eggs in each gram of feces following the formula: nº of eggs of the sample = nº of eggs found in the blade X factor 24 (the factor varies depending on each Kit). The Kato-Katz kit was gently donated by the Osvaldo Cruz-Biomanguinhos Foundation, Rio de Janeiro-RJ (Brazil).

2.6 Collecting adult worms from the hepatic portal system

The adult worms were collected from the mice via perfusion of the hepatic portal system (obtained by section), with phosphate buffered saline 0,85% on the 48th and 85th days after the infection. The reduction percentage (%) of the parasitic charge was measured comparing the number of mated adult worms obtained in each experimental group and its corresponding control, (infected only), according to Fonseca's formula (2004) GR=RGC – RGE x 100/RGC, in which GR is the degree of reduction, RGC is the number of worms collected from the control group, and RGE is the number of worms collected from the experimental group.

2.7 Statistical analysis

The results were expressed as means and standard deviations. Data from pairs of groups were analyzed using the Mann-Whitney U test; and data from multiple groups were analyzed using the ANOVA test followed by a multiple comparison test (Bonferroni's Multiple Comparison Test) (Prism 5.0 software).

3. Results

3.1 Evaluation of eggs per gram of feces on the 47th day after infection

In figure 1, the immunized with PNP1 enzyme/infected group presented significant decrease in the number of eggs per gram of feces when compared with the infected only group. Also the immunized with HGPRT enzyme/infected group showed significant decrease of eggs per gram of feces when compared with the infected only group. The immunized with ADK enzyme/infected group showed a non-significant decrease of eggs per gram of feces compared with the infected only group.

3.2 Number of mated adult worms recovered on the 48th day after infection

The mated adult worms recovered from groups: only infected, immunized with PNP1 enzyme/infected, immunized with HGPRT enzyme/infected and immunized with ADK enzyme/infected are depicted in figure 2, along with their respective percentages (%) of reduction of parasite load , calculated according to Fonseca et al., 2004. The average number of mated adult worms recovered from the infected group was 85, whereas the immunized with PNP1 enzyme/infected group presented an average number of 72. As for the immunized with HGPRT enzyme/infected group, the average number of mated worms was 62, and 77 for the immunized with ADK enzyme/infected group. The percentage reduction in the parasite load in the group of animals immunized with PNP1 enzyme /infected group was of 25%, if compared to the infected only group, whereas the group of animals immunized with HGPRT enzyme/infected showed a percentage reduction of 27 % of parasite load, if compared to the infected only group. In the group of animals immunized with ADK enzyme/infected, the percentage of reduction was 9,41 % over the infected only group.

3.3 Evaluation of eggs per gram of feces on the 84th day after infection

In figure 3, the immunized with the PNP1 and HGPRT enzymes/infected groups showed a non-significant decrease of eggs per gram of feces, compared with the infected only group.

3.4 Number of mated adult worms recovered on the 85th day after infection

The mated adult worms recovered from groups: only infected, immunized with PNP1 enzyme/infected, immunized with HGPRT enzyme/infected and immunized with ADK enzyme/infected are depicted in figure 4, along with their respective percentages (%) of reduction of parasite load , calculated according to Fonseca et al., 2004. The average number of mated adult worms recovered from the infected group was 92, whereas the immunized with PNP1 enzyme/infected group presented an average number of 49. As for the immunized with HGPRT enzyme/infected group, the average number of mated worms was 71**,** and 67 for the immunized with ADK enzyme/infected group. The percentage reduction in the parasite load in the group of animals immunized with PNP1 enzyme /infected group was of 50%, if compared to the infected only group, whereas the group of animals immunized with HGPRT enzyme/infected showed a percentage reduction of 19 % of parasite load, if compared to the infected only group. In the group of animals immunized with ADK enzyme/infected, the percentage of reduction was 27 % over the infected only group.

4. Dicussion

Schistosomiasis, a disease caused by the trematodes of the *Schistosoma* genus, affects about 240 million people worldwide. Even though the existence of drugs against adult worms, there is deficiency of drugs which can control the morbid effects of infection or even vaccines which can control the parasite's dissemination. Although the Health Ministry has made available a vaccine for tests in humans, in 2012, its efficiency is still debatable. During infection, the immune response of the host against cercariae and schistosomules is initially mediated by Th1 cells. With the spread of infection, response is modulated by Th2 cells induced particularly during the formation of granulomas generated by egg antigens retained in the tissues of the host. (McManus & Loukas, 2008). The PZQ is currently the only effective drug against all *Schistosomas* (WHO, 2012) and its use in populations has presented some problems such as reduction of therapeutic efficacy in patients from different regions such as Senegal and Egypt. (Gryseels et al., 2001; Ismail et al., 1999). Thus, the search for new therapeutic tools is necessary, such as effective ways of immunization. The main characteristic of an ideal vaccine for schistosomiasis is the ability to decrease the parasite load, being the migrating schistosomula the main target (Wilson & Coulson, 2006). However, in the case of schistosomiasis mansoni, the complexity of the parasite's life cycle within its hosts, as well as the variety of immune responses induced by them, hampers the development of an effective vaccine (Dupre et al., 2001). The mated adult worms of *S. mansoni* live in the venules of the hemorrhoidal plexus and the portal system, (Henri et al., 2002) and in these locations, females make oviposition of around 300 eggs per day and approximately 20% of them go toward the intestinal lumen, while the rest is mostly stuck in the intestine, spleen and liver of the host, causing inflammatory reactions. (Lenzi et al., 1987). This inflammatory process, triggered mainly by 2antigens released from eggs, promotes increased cellular infiltration, directing inflammatory cells to form granulomas around eggs trapped in the tissues. The decrease in the number of eggs is very important, as the granulomas are caused mainly by immune responses against soluble egg antigens (SEAs) and also because a lesser number of eggs being deposited in tissues can cause a reduction of granuloma (Helmy et al., 2009), causing a possible morbidity reduction of this pathogenesis. Thus, our results showed, on the 47th day after infection, a significant reduction in the number of eggs per gram of feces of animals which were immunized/infected with the PNP1 and HGPRT enzymes (Fig. 1), suggesting that these enzymes can interfere with the process of oviposition by females or be related to the reduction of adult worms. The activation of the immune response during infection may favor the control of the infectious agent. Therefore, it is of great value to note whether the modulation of immunological parameters interfere in the infectious process, such as by analysis of parasite load and of the cellular profile involved in the inflammatory process triggered during infection. In the work of Neris (2013) it was demonstrated that the cited enzymes are able to stimulate the production of immunoglobulins of the IgG, IgG2a and IgE classes. Our results showed that at 48th and 85th days after infection, all groups which were immunized with the studied enzymes showed a reduction in parasite load (Fig. 2 and 4). Therefore, our results corroborate with other studies which used different proteins of *S. mansoni* through immunization in animal models, yielding promising results. (Matsumoto, et al.,1988**;** Pearce et al.,1988; Correa-Oliveira et al.**,** 1989**;** Moser, et al., 1991; Porchet et al., 1994; Da'Dara, et al., 2001; AL-sherbiny et al., 2003; Varaldo et al., 2004; Fonseca,et al., 2005). It is observed that our immunization with the enzymes promoted a possible immunoreactivity, favoring the reduction of parasitic load. In face of these facts, emerged the

hypothesis that the proposed enzymes, despite being related to the parasite's DNA, can be expressed in the *S. mansoni* membrane and secreted by some pathway. It is necessary, however, further studies in order to fully comprehend the immune mechanism from the mentioned enzymes in this experimental model.

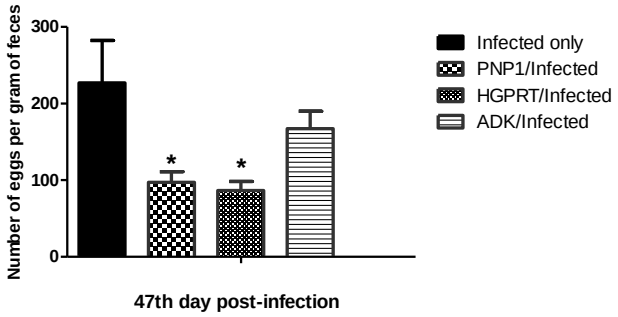

Figure 1 - Number of eggs per gram of feces on the 47th day after infection. Data represent mean ± EPM (n= 10 animals) of 2 independent experiments. The symbol * ($p < 0,05$) represents a significant difference between the results obtained from the immunized/infected groups when compared to the infected group, using the nonparametric one-way ANOVA Tukey's test.

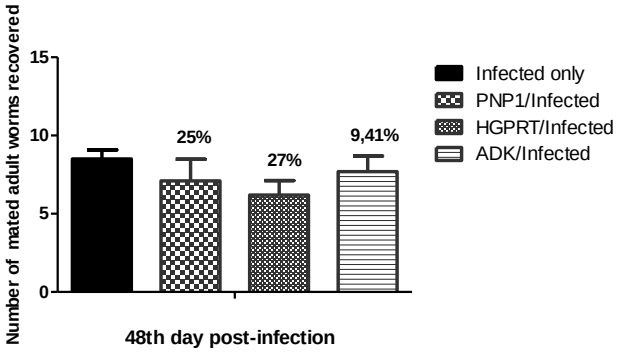

Figure 2. Number of mated adult worms recovered in each group, along with their respective percentages (%) of reduction of parasite 48th day after infection. Data represent mean ± EPM (n= 10 animals) of 2 independent experiments, using the nonparametric one-way ANOVA Tukey's test.

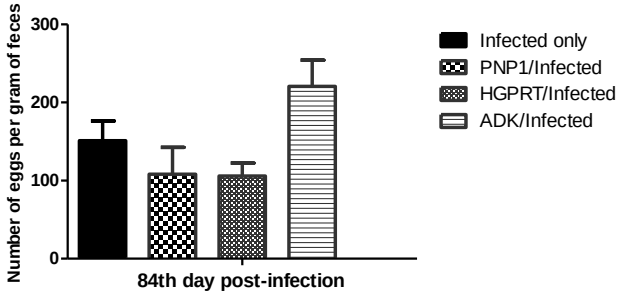

Figure 3. - Number of eggs per gram of feces on the 84th day after infection.
Data represent mean ± EPM (n= 10 animals) of 2 independent experiments, using the nonparametric one-way ANOVA Tukey's test.

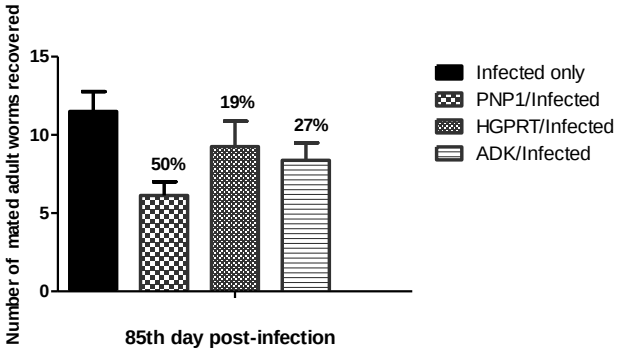

Figure 4. Number of mated adult worms recovered in each group, along with their respective percentages (%) of reduction of parasite 85th day after infection. Data represent mean ± EPM (n= 10 animals) of 2 independent experiments, using the nonparametric one-way ANOVA Tukey's test.

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