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Schistosoma mansoni: PROTECTIVE IMMUNITY IN MICE CURED BY CHEMOTHERAPY AT THE CHRONIC PHASE OF THE DISEASE

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SUMMARY

Aiming at demonstrating a decrease of acquired immunity after chemotherapeutic cure, a group of mice was infected with 25 **Schistosoma mansoni** cercariae (LE strain). A part of these animals was treated with 400 mg/kg oxamniquine, at 120 days after infection. Challenge infections were carried out at 45, 90 and 170-day-intervals after treatment (185, 210 and 290 days after primoinfection, respectively). Recovery of worms at 20 days after reinfections showed that a residual immunity remains up to 90 days after treatment, and disappears at 170 days after cure.

Using the ELISA method, it was possible to detect a decrease of antibody levels (total IgG) in the treated group, when antigens from different evolutive stages of **S. mansoni** were used.

The epidemiological implications of the present results, and the possible mechanisms involved in the decrease of acquired immunity after treatment are discussed.

KEY WORDS: Schistosoma mansoni; Chemotherapy, Residual immunity; Concomitant immunity.

INTRODUCTION

There has been long since several laboratorial and epidemiological studies bore evidence that man, as well as other animals, are able to acquire resistance to reinfections with parasites pertaining to the genus **Schistosoma**. FUJINAMI[18] was the first author to demonstrate this resistance to reinfections, using horses infected with **Schistosoma japonicum**. Several other authors corroborated these findings[19,34,43], all of them using **S. japonicum**. FARLEY et al.[17] demonstrated this acquired resistance in monkeys infected with **Schistosoma spindale**, whereas KAGAN[26] did the same using mice infected with **Schistosoma douthitti**.

In relation to **Schistosoma mansoni**, OLIVER & SCHNEIDERMANN[33] AND STIREWALT[41] were the first ones to demonstrate this kind of acquired resistance. They verified that primoinfection was partially capable of protecting mice against reinfection with cercarie. Posterior works showed this type of partial protection, according to the review carried out by DEAN[12]. SMITHERS & TERRY[40] introduced the idea of "concomitant immunity" in order to describe the ability of adult **S. mansoni** worms to survive in the blood stream of their hosts, in the presence of immunological mechanisms that act against the immature forms of reinfections. In spite of the fact that immunity depends on the presence of adult worms to be effective, and although it is well known that treatment "per se" causes a decrease in the antibodies against **S. mansoni** antigens[9,31], the time of permanence of protective immunity in treated and cured hosts is still an important ques-

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tion to be answered. DOENHOFF et al[15] and ANDRADE & BRITO[1] showed the permanence of this immunity in mice treated at the acute phase of the disease. Nevertheless, it is well stablished that the concomitant immunity is expressed in its magnitude at the chronic phase[40]. However, very few works have been carried out at this phase of the disease, which involves most of the infected people of endemic areas. The literature shows only one paper dealing with the residual immunity after chemotherapeutic cure in mice treated at the chronic phase of the disease (WARREN et al[45]). The authors used mice treated 32 weeks after infection, and challenged 6 weeks later, which showed a decrease (50%) in the number of worms in relation to the control. Nevertheless, the main emphasis of Warren's work deals with immunopathology as a result of reinfection.

The present study aims at determining, in regard to time, the permanence of protective immunity in mice treated and cured at the chronic phase of the disease, and challenged up to 170-day-intervals after treatment.

This approach, taking into account the due proportions between mouse and man is important when the difficulties in the evaluation of the damage caused by reinfections in individuals of endemic areas are considered. It is worthwhile to note that chemotherapy for mass treatment is the main prophylactic measure used in Brazil, with thousands of treated patients that run the risk of being reinfected.

MATERIAL AND METHODS

Female adult albino Swiss mice (**Mus musculus**), undefined breed, weighing 20 g, approximately, were used.

Primoinfection was performed by subcutaneous route, with ± 25 **S. mansoni** cercariae, LE strain, from Belo Horizonte (MG, Brazil), and kept over 25 years at the laboratories of the Schistosomiasis Research Unit (Federal University of Minas Gerais, Brazil), with hamsters (**Cricetus auratus**) and **Biomphalaria glabrata** snails.

Simultaneously, another group of uninfected mice, belonging to the same batch, was left as control. At 120 days after infection, a number of animals was treated with 400 mg/kg oxamniquine (MANSIL® , Pfizer Laboratories), by oral route.

Forty-five and 90 days after treatment 12 animals from Group 1 (GI - infected and treated mice), 12 animals from Group II (GII-infected and untreated mice) and 15 animals from Group III (GIII-control mice) were separated and challenged with ± 80 cercarie by transcutaneous route, according to the technique by BARBOSA et al[5]. Mice were anesthetized with 0.05 ml Diempax® (diazepam 10mg/ml), and 5 min later with 0.1 ml Nembutal® , corresponding to about 35 mg/kg sodium pentobarbital, both of them by intraperitoneal route. At 170 days after oxamniquine treatment, mice from groups I, II, and III were divided into two subgroups. The first subgroup received a 80-cercaria-burden, and the second a 15-cercaria-burden. The same procedure being maintained for the respective control animals. Twenty days after the challenge infection, mice were sacrificed and perfused for worms, according to the technique prescribed by PELLEGRINO & SIQUEIRA[35] . Systematic blood collections were carried out, starting from primoinfection until the last perfusion, in all the animal groups. In the collection performed before perfusions, the mice were anesthesized with ether and bleeded. The blood collections held at the intervals between primoinfection and perfusions were conducted in the ophthalmic plexus, with a Pasteur pipette.

The sera were submitted to the Enzyme Linked Immunosorbent Assay (ELISA), following the technique by TAVARES et al.[44]. For this test, five antigens from cercaria, adult worm, adult worm tegument, schistosomule and soluble egg antigen were used, at a concentration of 10 μg antigen/ml. Dilutions of 1/100 to 1/3200 were carried out. After a careful analysis of the dilutions used, dilution 1/3200 was chosen to be used for serological results.

For statistical analysis, the mean values obtained for different antigens were taken into account. Data were analysed by means of the analysis of variance method, with a significance minimum of $p < 0.05$.

RESULTS

WORM RECOVERY

When the worm recovery means related to the parasites from the challenge infection conducted at 45 days after treatment were considered, significant difference among the three groups was observed.

At 90 days after treatment, statistically significant differences could be seen between groups I and II, in relation to the respective control. At 170 days after treatment no significant differences could be detected between the treated groups (G-I) and the respective controls challenged with 15 and 80 cercariae (290 days after primoinfection). On the other hand, it was possible to verify the presence of concomitant immunity in group II (challenged with 15 and 80 cercariae), as well as a marked protection in the group challenged with 15 cercariae (66%), when compared with the group challenged with 80 cercariae (26%), both in relation with the control group. $P < 0.001$ (Table 1).

ELISA ASSAYS

TREATED GROUP (G-I)

In this group, a marked increase for the antibody levels could be observed up to 120 days after infection. A decrease in these levels was seen after treatment, this decrease being maintained even after the challenge infections carried out at 45 and 90 days after chemotherapy (Fig. 1).

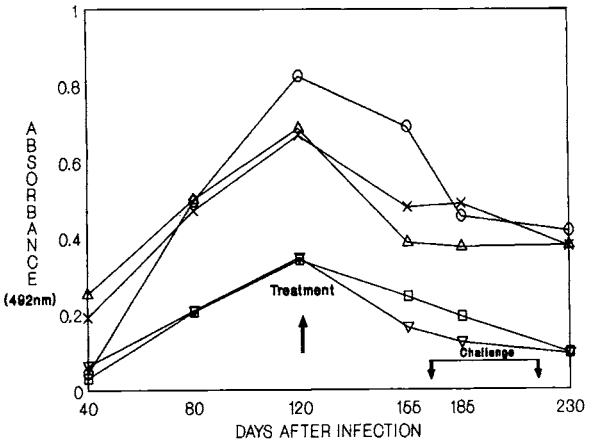

Figure 1 - Antibody levels (total IgG) in the serum of infected, treated, and challenged mice were measured by ELISA, against **S. mansoni** antigens.

Mice were infected (25 cercariae), treated with 400mg/kg of oxamniquine, orally at 120 days after infection, and challenged (80 cercariae) at 45 and 90 days after treatment (165 and 210 after the primary infection). Blood was collected 20 days after challenge infection. The antibody levels were measured for the following antigens: □ cercaria; ▽ schistosomule; △ adult worm; X adult worm tegument; O soluble egg antigen.

Figures 3 and 4 clearly show a statistically significant decrease of antibodies levels in treated group, when compared with previously infected and untreated group, at 170 days after treatment.

UNTREATED GROUP (G-II)

An increase of the antibody levels was also observed in this group, up to 120 days after infection. After this period, it was possible to not some stabilization of those levels, which was not altered even after challenge infections (Fig. 2).

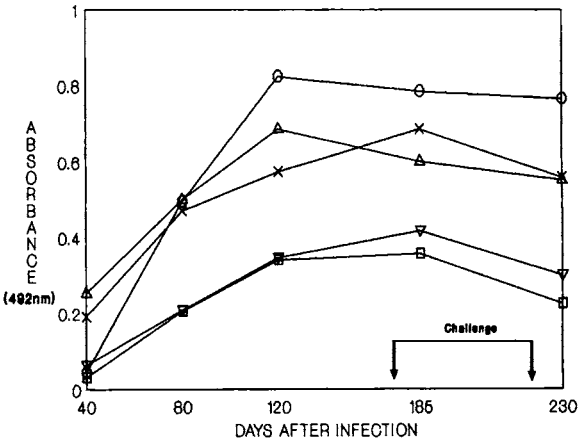

Figure 2 - Antibody levels (total IgG) in the sera of infected, untreated, and challenged mice were measured by ELISA, against **S. mansoni** antigens.

Untreated mice were infected (25 cercariae) and challenged at 165 and 210 days after the primary infection. Blood was collected at 20 days after challenge infection. The antibody levels were measured for the following antigens: □ cercaria; ▽ schistosomule; △ adult worm; X adult worm tegument; O soluble egg antigen.

DISCUSSION

Millions of people in different endemic areas in the world were submitted to efficacious chemotherapeutic treatment, and are still exposed to reinfection risks. Their acquired immunity would be of the concomitant type, that is, depending on the presence of adult worms to be able to stimulate immune response against the early forms of infections. So, it is of fundamental importance to know the kinetics of the decrease in this kind of acquired immunity after chemotherapeutic

cure. In spite of the relevance of the subject, it is surprising to verify the scarcity of research works conducted at the chronic phase of the disease, aiming to detect a decrease of the so called "concomitant immunity", after successful treatment. Under these special conditions, it is now necessary to use the expression "residual immunity" instead of concomitant immunity, since the adult worms were destroyed by drug action.

Brief comments on detection of protective immunity up to 42 days in treated and cured mice, at the chronic phase of the disease were made by WARREN[45]. He did not continue his observations on this subject, at subsequent dates and focused this theme basically under the anatomopathological view-point.

The present study demonstrates the permanence of protective immunity up to 90 days after parasitological cure by chemotherapy. It is also evident the disappearance of the acquired immunity at 170 days after treatment (Table 1). The influence of the cercarial burden of a challenge infection on the level of protection obtained is of paramount importance. As can be seen in Table 1, the 15-cercaria-burden in the animal group challenged at 170 days after treatment resulted in 66% protection, whereas the 80-cercaria-burden provided 26% protection only. It can be speculated that the 80-cercaria-burden has overloaded the immunological mechanisms responsible for protection at 290 days after primoinfection (170 days after treatment). It is worth mentioning that such a burden (80 cercariae) for a diminute animal as the mouse (about 30 g) corresponds to a 160-thousand-cercaria-load for a human weighing about 60 kg, a very high worm burden that could exhaust the protective capacity of the specific immune response. Figures 1, 3 and 4 show the decrease, after chemotherapeutic cure, in immunoglobulins connected with several antigens of the parasite, corroborating the data obtained by other authors[9,31,42].

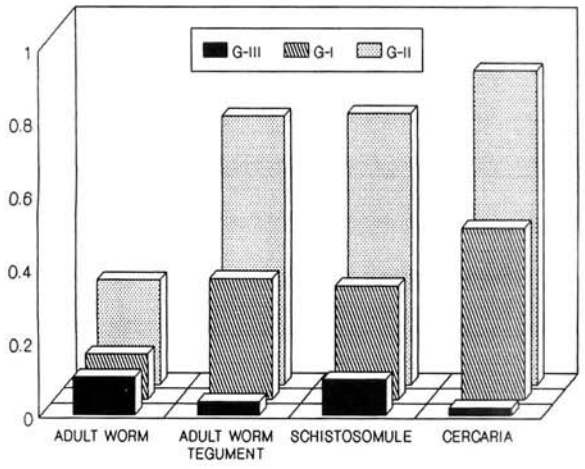

Figure 3 - Antibody levels (total IgG) against **S. mansoni** antigens were measured by ELISA in the sera of treated (G-I), with concomitant immunity (G-II) and control mice (G-III). All the animals were challenged with 80 cercariae.

G-I - Mice treated at 120 days after primoinfection (25 cercariae), and challenged at 170 days after treatment. G-II - untreated mice challenged at 290 days after primoinfection. G-III - control mice. Blood was collected at 20 days after challenge infection.

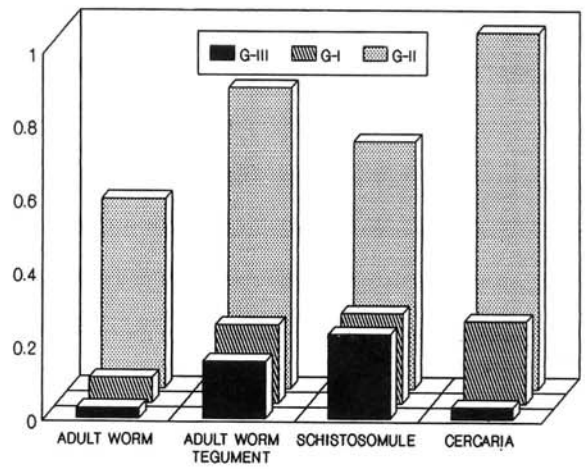

Figure 4. - Antibody levels (total IgG) against **S. mansoni** antigens were measured by ELISA in the sera of treated (G-I), with concomitant immunity (G-II) and control mice (G-III). All the animals were challenged with 15 cercariae.

G-I - Mice treated at 120 days after primoinfection (25 cercariae), and challenged at 170 days after treatment. G-II - untreated mice challenged at 290 days after primoinfection. G-III - control mice. Blood was collected at 20 days after challenge infection.

It is evident the efficacy of the dosage 400 mg/kg oxamniquine, which provided cure for all the animals belonging to group I (no worms from primoinfection could be recovered). Further it is quite clear that the remaining acquired immunity after treatment does not disappear suddenly, since it acts up to 90 days after cure (Table 1).

TABLE 1

Recovery of worms in mice following **Schistosoma mansoni** infection, after treatment and challenge.

INTERVAL BETWEEN TREATMENT AND CHALLENGE (DAYS)	EXPERIMENTAL GROUP		NUMBER OF CERCARIAE AT THE CHALLENGE INFECTION	MEAN OF WORMS		% OF PROTECTION RELATED TO THE CONTROL ANIMALS
				PRIMO INFECTION (ADULTS)	IMMATURE (20 DAYS) (X ± SD)	
45	INFECTED AND TREATED	G-I	80	0	20.82 ± 8.68	30%
	INFECTED	G-II	80	6.0	11.16 ± 7.98	63%
	CONTROL	G-III	80	—	29.84 ± 11.7	—
90	INFECTED AND TREATED	G-I	80	0	19.16 ± 4.15	26%
	INFECTED	G-II	80	10.3	17.25 ± 9.38	33%
	CONTROL	G-III	80	—	25.83 ± 6.04	—
170	INFECTED AND TREATED	G-I	80	0	27.12 ± 10.45	N.S.
	INFECTED	G-II	80	5.1	19.36 ± 7.21	26%
	CONTROL	G-III	80	—	26.07 ± 6.14	—
170	INFECTED AND TREATED	G-I	15	0	5.37 ± 2.26	N.S.
	INFECTED	G-II	15	5.4	1.83 ± 1.19	66%
	CONTROL	G-III	15	—	5.38 ± 2.36	—

Primoinfection was performed with 25 cercariae. Percentages showed are statistically significant at $P < 0.05$.
N.S. = Not significant

In the experimental Schistosomiasis, several mechanisms that could participate in the concomitant immunity have been described. So, even the hemodynamic alterations related to the portal circulation, due to the lesions produced by the disease, could provoke the return to the lungs of some young worms (from reinfections), that had recently arrived in the liver. In this case, a number of these worms would be destroyed by inflammatory reactions in the alveoli[13,46]. The amelioretion of the lesions could, in part, explain the decrease of protection after chemotherapy, since more recently it was showed a significant regression of the fibrous tissue after cure[3]. This decrease of fibrosis would be, mainly, periportal and connected with a recent collagen tissue, as occurred in the present study. This fact could lead to hemodynamic normalization in the portal-cava system. Therefore, there would have a marked decrease in the migration of immature worms from the liver to the lungs.

On the other hand, the allergic reaction at the skin, at the moment of cercarial penetration in reinfections (anaphylaxis or type I-hypersensitivity) has been described as an important mechanism in acquired immunity[4,8,20,21,25,32,38,39]. It is possible to infer that this kind of mechanism seems to have no participation in the decrease of immunity as showed in our results, since in humans, individuals that were cured by chemotherapy continue presenting positive intradermalreaction (Type I - hypersensitivity) after treatment[36].

Otherwise, the decrease of immunoglobulins (mainly IgG2a) that acted against schistosomules[7,22] could also explain in part the decrease of immunity after cure, as can be seen in this study. Figures 1, 2, 3 and 4 show (chiefly Figures 3 and 4, 170 days after treatment) a homogeneous decrease of antibodies against several **S. mansoni** antigens, in the treated and cured group.

As far as the phylogenetic differences and body weight are concerned, it is important, once more, to keep the due reservation, when one wants to extrapolate the results obtained in mice to human populations. Nevertheless, there are epidemi-

ological evidences that support the hypothesis related to the occurence of a similar fact in human populations submitted to chemotherapeutic mass treatment. Several research works[2,6,10,11,14,23,24,28,30,37] showed that the rates of the severe forms of the disease can decrease, markedly, after chemotherapeutic treatment although the prevalence of the disease reaches in few years the levels recorded before treatment. Since the transmission process of the disease remains after treatment, probably the individuals cured by chemotherapy are able to acquire lower worm burdens, due to residual immunity, these new burdens stimulating the restablishment of the concomitant immunity.

RESUMO

Schistosoma mansoni: **imunidade protetora em camundongos curados por quimioterapia na fase crônica da doença**

Para evidenciar a queda da imunidade adquirida após cura quimioterápica, um lote de camundongos foi infectado com ±25 cercárias de **S. mansoni** (cepa LE). Parte destes animais foi tratada aos 120 dias com 400 mg/kg de oxamniquina. Foram feitos desafios em intervalos de 45, 90 e 170 dias após o tratamento (185, 210 e 290 dias após a primoinfecção, respectivamente). A recuperação aos 20 dias após as infecções desafio, dos vermes das reinfecções mostrou que a imunidade residual persiste até os 90 dias após o tratamento e desaparece aos 170 dias após a cura.

Com o soro dos camundongos, através do método de ELISA, evidenciou-se uma queda nos níveis de anticorpos (IgG total) no grupo tratado em relação a vários antígenos de estágios evolutivos do **S. mansoni.** As implicações epidemiológicas dos presentes resultados e os possíveis mecanismos envolvidos na queda da imunidade adquirida após tratamento são discutidos.

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