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Effects of immunosuppression on the course of Schistossomiasis mansoni infection in the Laboratory Rat·

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RESUMO

Ratos albinos, experimentalmente infectados com *Shistosoma mansoni*, foram submetidos a alguns esquemas de tratamento prolongado com a Azatioprina (uma droga imunossupressora), visando a modificar o curso da infecção. O composto foi administrado duas vezes por semana, com o tratamento começando três dias antes, ou 15, 21 e 30 dias após a infecção, até o sacrifício dos animais. O efeito imunossupressor do tratamento foi avaliado medindo-se as respostas celular e humoral dos roedores às hemácias de carneiro. O composto não altera a infectividade, como se verifica pela carga de vermes recuperados no 25o. dia de infecção. Contudo, um significante retardamento na "autocura" (eliminação expontânea e progressiva dos esquistossomas) foi observado nos ratos tratados três dias antes e naqueles imunossuprimidos aos 15 e 21 dias após a infecção. Nos tratados 30 dias após não houve retardamento no fenômeno de "autocura". Não se observaram modificações nos tamanhos dos esquistossomos recuperados. Por outro lado, detectou-se maior migração de vermes para as veias mesentéricas dos animais imunossuprimidos.

Este modelo de imunossupressão tem a vantagem de ser iniciado ou terminado a qualquer tempo da infecção experimental.

INTRODUCTION

The laboratory rat presents peculiar host-parasite relationship following *Schistosoma mansoni* infection. Although easily infected, this rodent shows a gradual elimination of worms, beginning by the 4 th week after cer-

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carial exposure or inoculation. The loss of worms resembles the so-called "self-cure phenomenon" (KRAKOVER *et alii*,[2] STIREWALT *et alii*,[14] STIREWALT & HACKEY,*15*, SMITHERS & TERRY,[13] MADDISON *et alii*[4] & PHILLIPS *et alii*[10]). Moreover. the survining worms do not reach full development, but they remain in the liver blood vessels and producing few eggs, usually infertile (RITCHIE *et alii*[12]).

A good advance for the understanding of the "self-cure phenomenon" was obtained through the infection of thymectomized and irradiated rats, and a very significant difference between worm recoveries from immunosuppressed and control animals was detected (CIOLI & DENNERT[1]). Previously, another trial on this subject was performed (MADDISON *et alii*[5]) with drainage of thoracic duct, and the use of anti-lymphocytic globulin and azathioprine. This drug was administered at the dose level of 3 mg per rat, from day-8 to O (infection day), daily, but no differences where obtained between the worm burden from treated and control animals necropsied four weeks later.

The present study demonstrates that the course of experimental schistosomiasis, in the laboratory rat, can be significantly altered by a prolonged treatment with azathioprine, performed until the necropsy day.

MATERIALS AND METHODS

Cercariae and infection

Cercariae (LE strain) used in this experiment where obtained from laboratory reared and infected *Biomphalaria glabrata*. About 500 cercariae were injected subcutaneously with a Cornwall syring, suplied with a 10 gauge needle, after cercarial concentration (PELLEGRINO E MACEDO[8]).

Rats

Outbred albino rats (150 - 200g) were selected for the experiments. After cercarial injection, they were maintained with the conventional food pellets and water *and libitum*.

Immunosuppressive drug

Experiments were carried out with azathioprine (AZT). The drug was given to a group of rats, from the 3 th day before infection until the necropsy day, at the dosage of 76 mg/Kg, twice a week, subcutaneously. Since a high mortality was observed in other experiments with that dose, other groups of animals received 38 mg/Kg, according to the same schedule of treatment, i. e., starting 3 days before, and 15, 21 and 30 days after infection. These groups were treated continuously until the end of experiments, except for the control groups, which received no treatment.

Worm recovery

The technique of PELLEGRINO & SIQUEIRA (9) was fallowed with minor modifications. A pipetting machine for saline injection into the aorta was used. A first perfusion of mesenteric vessels was done and, after that, liver perfusion was obtained by injecting saline into the hepatic sinus. The worms were then washed with saline, removed to flashs with 10% formalind and, later on, counted undera dissecting microscope.

Worm measurement

Camera lucida drawings of the worms were performed for further measurements.

Evaluation of immune response

Cellular immune responses to sheep red blood cells (SRBC) were evaluated by the footpad swelling technique (MILLER *et alii*[7]). Sensitizing and eliciting doses of 1 x 10^8 SRBC were given to each animal. As far as tests with *S. mansoni* saline extract were concerned, only the eliciting dose (48 µg protein) was injected into the foot of rats previously infected with the parasite, following the same procedure.

The humoral response was evaluated by the hemagglutination technique (LIMA & DIAS DA SILVA[3]). The titles were considered as the reciprocal of serum dilutions.

RESULTS

Efecctes of treatment with azathioprine on cellular and humoral responses.

Data are summarized in Table 1.

Significant differences ($p < 0.5$) between cellular immune responses ot treated and control groups were by measuring the footpad swelling. SRBC and *S. mansoni* extract were used as antingens. Statistical analyses of the titles of hemagglutination were by tests. The titles were found lower ($p < 0.05$) in the treated group, indicating a partial suppression of the humoral immune response.

Effects of treatment (started 3 days before infection) on worm burden

Data are summarized in Table II.

A group of 21 infected rats (about 500 cercariae per animal) was treated with AZT (38 mg/Kg), twice a week, with treatment starting 3 days before cercarial inoculation and prolonged until the necropsy day. Another group of 21 infected rats,. receiving no treatment, served as the control.

Ten animal of each group were sacrificed after 25 days of infection, in order to detect possible differences in worm recoveries. Worm burdens from the two groups were very close at that time (48.1 ± 27.8 and 42.2 ± 28.2, respectively). However, perfusions of the remaining animals performed at day 38 showed a significant difference (15.6 ± 11.7 versus 5.7 ± 5.2), with $p < 0.05$. Besides, treated animals showed and average of 9.3% of worms in the mesenteric vessels, against 1.4% at the same time in the control group. As can be seen, these percentages differ signicantly from each other.

Effect ot treatment (started 15, 21 ans 30 days after infection) on worm burden

Data are summarized in Tablle III.

The elimination of parasites could be delayed by the drug, provided drug administration started two or three weeks after infection. However, the compound did not alter the rate of worm elimination, when given after the fourth week.

Worm measurement

Measurements of schistosomes recovered from animals whose treatment started 3 days before infection (172 worms), did not differ (2.7 ± 0.8 mm and 2.8 ± 0.6 mm, respectively) from each other.

DISCUSSION AND CONCLUSIONS

Schistosoma mansoni infection of the laboratory rat follows a peculiar pattern. This rodent is easily infected with the parasite, although the number of worms recovered at the 4 th week after cercarial exposure is generally lower than that obtained from more susceptible hosts, such as the mouse and the hamster (STIREWALT &HACKEY [15]). In the rat, after the 4 th week of infection, the worm burden declines rapidly, and then a lower and more stable number of worms is found (PHILLIPS &COLLEY [11]).

In rats with prolonged immunosuppression produced by azathioprine, the worm burden remains at the same levee of that in non-immunosuppressed rats, until the 4 th week. However, a significant delay in the elimination of the parasites is observed subsequently (Table II). the "self-cure phenomenon" presented by those animals can also be delayed easily, if drug administration is initiated two or three weeks after cercarial inoculation. The compound does not alter the rate of worm elimination, it is given after 4 th week (Table III). Thus, it can be concluded that the antigenic stimuli originated from maturing larvae, which occur prior to the 30 th day of infection, are necessary to worm rejection.

These findings indicate participation of immune response in this phenomenon. In fact, prolonged administration of azathioprine reduced the cellular immunoresponse to *S. mansoni* antigens in infected rats, as well as both humoral and cellular immunoresponses to SRBC in uninfected animals (Table I).

The results presented here are consistent with those obtained by CIOLI &DENNERT [1] in thymectomized and irradiated rats, as far as higher worm burdens following lower titles of specific antibodies against the parasites are concerned.

Some authors (VERNES *et alii*[16]) suggested that the cellular immune response plays an important role in the "self-cure." By the system used here, it was not possible to evaluate neither humoral nor T cell medited responses, since azathioprine inhibits both (MAKINODAN *et alii*[6]).

The present results obtained with azathioprine markedly differ from those reported by other investigators (MADISON *et alii*[5]), who did not observe changes in the worm burden of immunosuppressed rats. This difference may be in part methodologic, since the above mentioned investigators treated the animal only from day -8 to the infection day.

The possibility of a direct effect of the drug on the worms was also examined. This is unlikely, since necropsies performed 25 days after infection showed similar worm burdens in both treated and untreated animals (Table II). Besides, it is widely know that active substances against. *S. mansoni* are capable of producing hepatichifts, and the azathioprine-treated animals showed higher percentages of worm in mesenteric vessels, when compared with the control (Table IV). No differences in worm size from different

TABELA I: Effects of prolonged treatment with azathioprine (doses of 38 mg/Kg) on cellular and humoral responses to SRBC and *Schistosoma mansoni* worm extract.

Antigen	Footpad swelling (mm)			Hemagglutination titles		
	Treated	Control	p	Treated	Control	p
SRBC	0.08	0.80	<0.05	140	355	< 0.05
S. mansoni extract	0.06	0.46	< 0.05			

TABLE II: Effects of prolonged treatment with azathioprine (doses of 38mg/Kg) on worm burden at 25 and 38 days after infection with 500 cercariae per animal. Drug administration was started 3 days before *Schistosoma mansoni* infection.

Groups	No. of animals	Days after infection	Mean worm burden and standard deviation	p
Treated	10	25	48.1 ± 27.8	NS
Control	10	25	42.2 ± 28.2	
Treated	11	38	15.6 ± 11.7	< 0.05
Control	11	38	5.7 ± 5.2	

NS – not significant

TABLE III: Effects of prolonged treatment with azathioprine (doses of 38 mg/Kg). Drug administration started 15, 21 and 30 days after infection in three groups of rats, previously infected with 500 cercariae. Necropsies were performed 38 days after infection.

Beginning of treatment (days after infection)	Number of animals	Mean worm burden and standard deviation	p
15	14	12.6 ± 7.3	< 0.05
30	14	4.6 ± 4.2	
			NS
Control	12	4.9 ± 4.7	
21	12	16.4 ± 15.1	
Control	18	8.3 ± 5.7	< 0.025

NS – not significant

TABLE IV: Effects of prolonged treatment with azathioprine (doses of 38 mg/Kg) on the migration of worm liver to mesenteric veins. Treatment was started 3 days before infection.

Groups	Number of rats	/Days after infection	Localization and number		%	p
Treated	10	25	H	477	9.54	
			M	4	0.08	
			P	0	0.00	
						NS
Control	10	25	H	418	8.36	
			M	0	0.00	
			P	4	0.08	
Treated	11	38	H	156	2.83	
			M	16	0.29	
			P	0	0.00	
						0.05
Control	11	38	H	62	1.12	
			M	1	0.02	
			P	0	0.00	

H – Intra-hepatic veins M – Mesenteric veis P – Portal vein NS – Not significant

groups of animals were seen either (Fig. I).

This model of AZT immunosuppression has the advantage to be initiated or ended at any time of experimental infection.

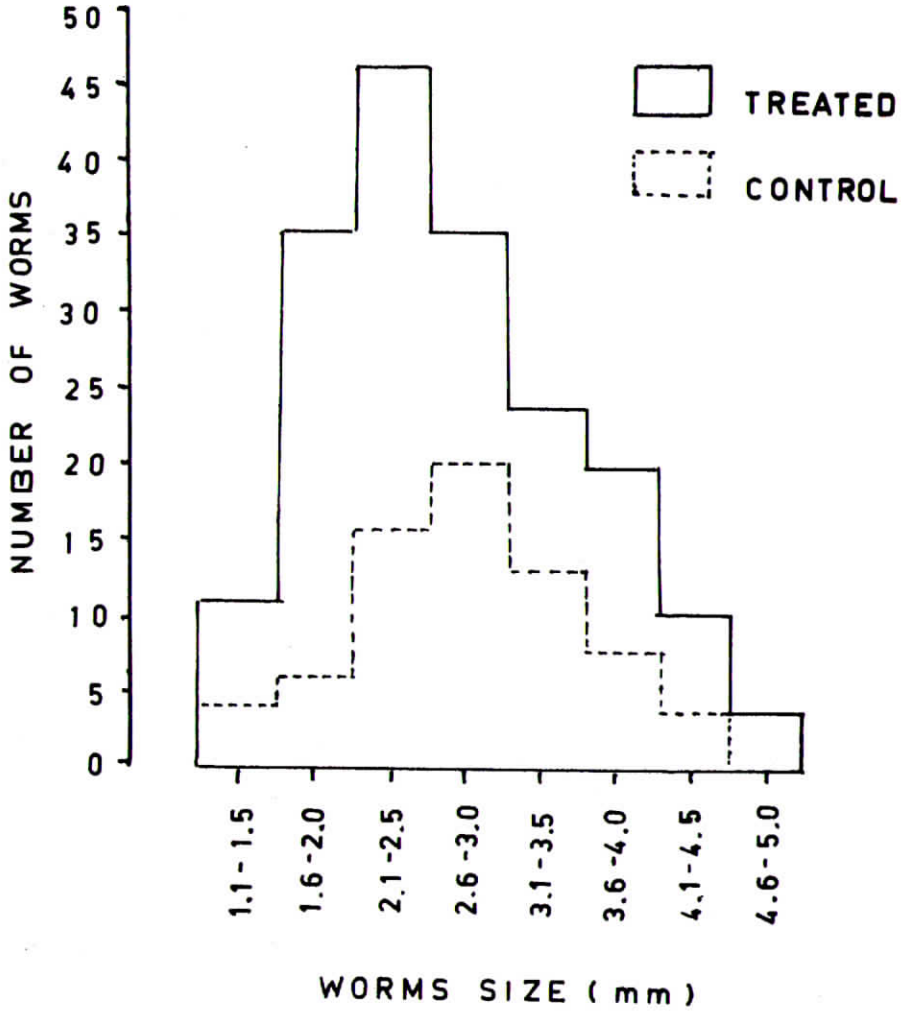

Fig. 1 – Recovery and mean worm lenghts for *Schistosoma mansoni* with 38 days. Treatment started 3 days before infection with azathioprine (doses of 38 mg/Kg).

SUMMARY

Albino rats, experimentally infected with *Shistosoma mansoni*, were submitted to several schedules of prolonged treatment with azathioprine (an immunosuppressive drug), aiming to change the course of infection. The compound was administered twice a week, and different treatments started 3 days before, or 15, 21 and 30 days after infection, until the sacrifice of animals. The immunosuppressive effect of these treatments was assessed by measuring cellular and humoral immunoresponses to SRBC. The compound did not alter infectivity as evaluated by the worm burden on the 25th day of infection. However, a significant delay in "self-cure" (progressive and spontaneous elimination of worms) was observed in rats when drug administration started prior to 30th day of infection. No changes in worm size, but different intravenous localizations of parasite were detected in the immunosuppressed rats. It was concluded that drug immunosuppression can prevent the worm rejection by the host, if treatment is started prior the beginning of rejection.

This model of immunosuppression has the advantage to be initiated or ended at any time of experimental infection.

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