

EVOLUTION OF SARCOMA 180 (ASCITIC TUMOR) IN MICE INFECTED WITH *SCHISTOSOMA MANSONI*.

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Mice infected with 60 cercariae of Schistosoma mansoni were more resistant to the sarcoma 180 ascites tumor. Tumor inoculation was performed 50 days after schistosoma infection and the animals were observed and weighed at 48 hours intervals for development and progression of malignancy. In infected mice the weight gain (ascites formation) started later and was shorter than in uninfected controls. Also, the number of tumor cells into the peritoneal cavity 72h after tumor implantation was shorter in infected group than in controls. This increased resistance against a transplantable tumor probably is related to the effect of endotoxin on tumoricidal activity of macrophages activated by the infection. The immunodepression induced by Schistosoma mansoni infection enhances the proliferation of endogenous bacteria increasing the amount of endotoxin absorbed from the gut.

Key Words: Experimental Schistosomiasis. Sarcoma 180. Schistosomiasis and cancer.

A relationship between *Schistosoma* infection and tumor development has been reported by some authors. Bladder cancer associated with *Schistosoma haematobium* was reported although an unequivocal statistical proof of this association has not been obtained⁴. A striking association between the giant follicular lymphoma of the spleen and schistosomiasis mansoni in hepatosplenic phase was found in Brazil¹⁷. A possible association between lymphoreticular tumors and schistosomiasis is described from Nigeria⁶.

In experimental studies, mice with *Schistosoma mansoni* infection were injected with 2-amino-5-azotoluene, a carcinogenic drug. The hepatomas appeared earlier and more frequently in infected animals than in respective uninfected controls⁵. Also, infected mice treated with a single dose of hycanthone developed hepatomas, whereas uninfected mice, treated with the same dose, did not⁷. However these observations showing a possible cocarcinogenic effect of *S. mansoni* infection were not corroborated by other investigators²¹. The association of schistosomiasis

and hepatocellular carcinoma is not proved¹⁹, although the geographical distribution of liver cancer in some countries corresponds to that of *S. mansoni* and *Schistosoma japonicum* infection¹⁵.

Finally, immunosuppression produced by *Schistosoma mansoni* infection is mentioned in several papers^{2 14 18}. As the immune system plays an important role on the development of neoplasia, we studied the evolution of a transplantable tumor in *Schistosoma mansoni* infected mice. It was demonstrated, by injecting sarcoma 180 cells into the peritoneal cavity of *S. mansoni* infected and uninfected control mice, that schistosoma infection is unfavourable to tumor development.

MATERIAL AND METHODS

Male, randombred, albino mice weighing 32-38 g were used in the experiments. The mice were infected by subcutaneous injection of 50-60 *Schistosoma mansoni* cercariae (LE strain). Fifty days after schistosoma infection the animals were injected with 2×10^6 cells of sarcoma 180, by the intraperitoneal route. The tumor cells were obtained from donor mice with ascitic tumor. Uninfected control mice, of the same age and weight, received the same dose of tumor cells.

The evolution of the tumor was followed up by: (a) weight gain after tumor implantation and (b) the counting of tumor cells into the peritoneal cavity 72 hours after tumor challenge. For evaluating weight gain, the animals were weighed at 48 hours time intervals. The difference between the initial weight (on

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the day of tumor implantation) and the weight after 48 h indicates the weight gain. To count tumor cells in the peritoneal cavity, groups of five mice were killed after ether anaesthesia and, after injection of a known volume of cold Ringer solution, the peritoneal cells were removed by aspiration. The cells were counted in a haematocytometer after dilution in a 0.01% gentian violet solution in 5% acetic acid.

RESULTS

After four days in control mice the weight gain was a linear function of the time after four days after tumor implantation. The incubation period (the time when the weight gain started) was 4.3 ± 1.2 days. In *Schistosoma mansoni* infected animals the weight gain started 6 days after tumor implantation and was lower than in controls (where the incubation period was 6.1 ± 1.8 days). Counting of tumor cells in peritoneal cavity 72h after tumor inoculation showed reduced development of tumor in infected mice. Data about the weight gain are summarized in the Figure 1 and the results of counting of tumor cells into the peritoneal cavity are shown in Table 1.

Table 1 – Evolution of sarcoma 180 in mice infected with 60 cercariae of *Schistosoma mansoni*: number of tumor cells into the peritoneal cavity 72h after implantation of 2×10^6 tumor cells on 50th day after infection (six mice per group).

Number of tumor cells $\times 10^6$ (mean \pm s)	
Control	Infected
13.35 \pm 3.11	7.85 \pm 2.23 *

* $p < 0.05$ (t test)

The number of peritoneal cells exudated 72 h after tumor challenge was greater in infected mice. In control mice 60% of the cells were mononuclear; in infected mice there was a great number of polymorphonuclear leucocytes (neutrophils and eosinophils). The survival time after tumor implantation was similar in the two groups (17.5 ± 2.2 days in controls and 16.7 ± 2.9 days in infected mice).

DISCUSSION

The evolution of sarcoma 180 in control mice occurred as it was expected: the ascite formation was a linear function of the time after four days of incubation period is the time interval between the tumor inoculation and the start of weight gain and is related to the number

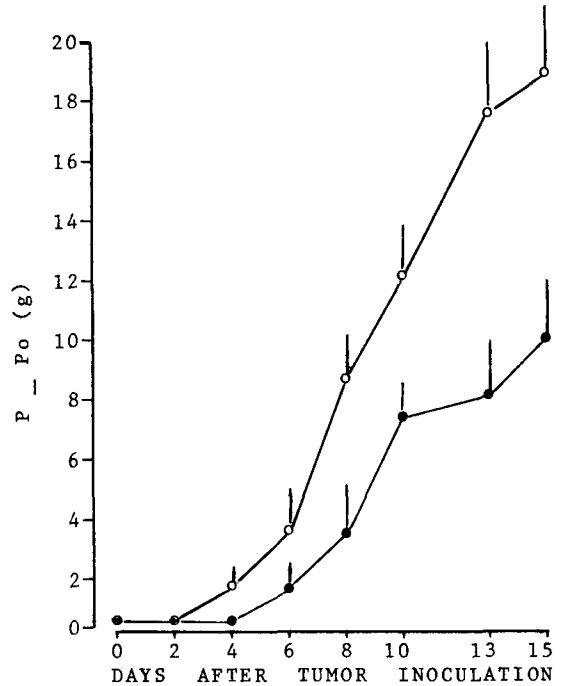


Figure 1 – Evolution of sarcoma 180 in mice infected with 60 cercariae of *Schistosoma mansoni* and inoculated with 2×10^6 tumor cells into the peritoneal cavity on day 50 after infection. Tumor evolution was evaluated by the weight gain ($P - P_0$; P_0 = weight on day of tumor implantation and P = weight in following days). The results are the mean \pm two standard deviations of ten mice per group.
o = control ● = infected with *S. mansoni*.

of tumor cells inoculated¹⁰.

In *Schistosoma* infected mice there was a reduction in tumor development, demonstrated by (a) an increased incubation period (higher than six days) and (b) a lowered number of tumor cells into the peritoneal cavity 72h after tumor implantation.

We have not yet an explanation for these observations but in other helminthic infections similar results were observed: mice infected with *Trichinella spiralis* are more resistant to the sarcoma 180^{10 13}. Also, in rats and mice infected with *Nippostrongylus brasiliensis* there is an increased resistance to transplantable tumors⁹. In *Trichinella spiralis* infected mice there is immunosuppression but there are evidences of macrophage activation and increased tumoricidal activity of peritoneal macrophages^{11 20}.

Schistosoma mansoni infection induce macrophage activation, beside the state of immunodepression and tumoricidal macrophages were demonstrated into the peritoneal cavity of three weeks

infected mice, after intraperitoneal challenge with soluble schistosoma antigens⁸. On the other hand, the immunosuppression that follows *Schistosoma* infection could induce increased proliferation of endogenous bacteria, increasing the absorption of endotoxin. It is known that endotoxin plays an important role on the tumoricidal activity of activated macrophages^{3 12}. Endotoxin induces activated macrophages to produce the tumor necrosis factor that kill tumor cells but not normal cells³. Thus, the peritoneal macrophages in infected mice could kill more tumor cells than in controls, producing reduction in the inoculum dose. In this way the number of tumor cells into the peritoneal cavity 72 h after tumor inoculation could be lower in infected mice than in controls.

In spite the increased resistance against the sarcoma 180 cells found in *Schistosoma mansoni* infected mice, the life span in control and infected animals was similar. The similitude between the survival time of infected and control mice could tentatively be explained by two kinds of immunosuppressor stimuli acting together: the *Schistosoma* infection and the tumor implantation. In fact mice infected with 60 cercariae start to die around the 60th day of infection. Also tumor injection induces reduction in the microbicidal activity of macrophages, enhancing opportunistic infection in tumor bearing mice¹⁶.

RESUMO

Camundongos infectados com 60 cercárias de Schistosoma mansoni tornaram-se mais resistentes ao sarcoma 180 na forma de tumor ascítico. A inoculação das células tumorais foi feita no 50º dia de infecção e a evolução do tumor foi acompanhada através da pesagem dos animais com intervalos de 48 horas. Nos camundongos infectados o ganho de peso (formação da ascite) começou mais tarde e foi menor do que nos controles não infectados. Também o número de células tumorais na cavidade peritoneal 72 horas após a implantação do tumor foi menor no grupo infectado. Este aumento de resistência a um tumor transplantável possivelmente está relacionado ao efeito de endotoxinas sobre a atividade tumoricida dos macrófagos ativadas pela infecção. A imunossupressão induzida pela infecção favorece a proliferação de bactérias da flora endógena aumentando a quantidade de endotoxinas absorvidas pelo intestino.

Key words: Esquistossomose experimental. Sarcoma 180. Esquistossomose e Câncer.

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