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Schistosoma mansoni: impairment of the cell-mediated immune response in mice

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SUMMARY

Skin-graft rejection in mice experimentally infected with *Schistosoma mansoni* is delayed when grafting is performed 60 days after the infection. In mice infected 30 days prior to the grafting, the grafts were rejected at the same time both in infected and in control animals. This observation indicates that impairment of the cell-mediated immune response occurs in mice with mature *S. mansoni* infections.

INTRODUCTION

A number of reports have suggested that cell-mediated immunity (CMI) is altered during infection with the helminth *S. mansoni*. Thus, on stimulation with soluble egg antigen, chronically infected mice show a decrease in delayed footpad swelling, production of lymphokines, macrophage inhibitory factor, and eosinophil stimulation promoter (Colley, 1975). The spontaneous diminution of cell mediated granulomatous lesions around *S. mansoni* eggs in mice with light infections, and the amelioration of hepatosplenic disease were demonstrated by Andrade & Warren (1964), Warren (1966), and Domingo & Warren (1969). These observations were in accord with the data of Boros, Pelley & Warren (1975) on the spontaneous modulation of granulomatous hypersensitivity in mice chronically infected with *S. mansoni*. Since the rejection of grafts is the expression of a CMI phenomenon it was considered of interest to study it in mice infected with *S. mansoni*.

MATERIALS AND METHODS

Mice. Random-bred white and inbred C57BL/10 adult, female mice were used. Infected and non infected white mice were used as receptors for the skin graft donated by the C57BL/10 animals.

S. mansoni. The LE strain was used. Each mouse was inoculated intraperitoneally (IP) with approximately eighty freshly emerged cercariae obtained from laboratory reared *Biomphalaria glabrata.*

Skin grafting. Both donor and receptor mice were anaesthetized with pentobarbital and a fragment of skin measuring 5 mm in diameter was removed from the shaved dorsal area of the receptor and replaced with a graft from the donor animal. The graft was carefully adjusted and the whole area was covered with a small piece of surgical gauze which was fixed in place with surgical tape. Seven days later the tape was removed and the graft exposed. At this time the graft was usually well fixed and without any sign of rejection.

The mice were observed daily for any sign of rejection or for the accidental removal of the graft. Mice which actively removed the graft were discarded. Up to 70% of both infected and non-infected mice actively removed the graft. This occurred most frequently in the first day after the removal of the protective tape (8 days of grafting). After this period of time active removal of the graft was minimal. Rejection signs were bleeding and shrinking of the graft with consequent detachment of its border. The day any of these signs were first noted was considered as the rejection day. Infected and grafted mice were killed a few days after rejecting the skin graft and perfused according to the technique described by Pellegrino & Siqueira (1956). Statistical analysis of the experimental data was conducted employing the Student's *t*-test and the correlation coeficient test (*r*).

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TABLE 1. Time elapsed between skin grafting and rejection of the graft in mice infected with *S. mansoni* (60 days of infection)

Days after skin grafting	Number of mice rejecting the graft	
	Infected	Control
9	0	5
10	3	22
11	3	—
13	6	—
14	5	—
15	5	—
16	2	—
18	1	—
Total	25	27

RESULTS

Both non-infected control mice and mice that had been infected with *S. mansoni* 30 days prior to skin grafting rejected the graft between days 8 and 10 after the skin transplant was performed. In Table 1 are the results obtained in the 60-day infected mice. Also in this experiment all control animals rejected the graft 10 days after grafting, whereas by this time only three out of twenty-five infected mice had done so. The highest number of infected mice rejected the graft between days 13 and 15. In one mouse the graft was eliminated only on day 18. The worm load was determined in nine mice which had rejected the graft between days 10 and 18. The results are shown in Table 2. Three mice which rejected the graft at day 10 had the lowest worm burden, whereas the mouse which eliminated the graft at day 18 had the highest worm load. The difference in rejection time in control and infected mice (Table 1) was highly significant ($P = 0{\cdot}001$), as well it was noted a positive linear correlation ($r = 0{\cdot}96$) between worm load and rejection time (Table 2).

DISCUSSION

The results reported in this paper show that cell-mediated immunity (CMI), as measured through rejection of skin graft, is altered in mice infected with the helminth *S. mansoni*.

CMI impairment was not demonstrated in mice infected 30 days prior to grafting, whereas it was evident in 60 day-infected mice. In the former animals the worms are still maturing and there is no oviposition. In the latter the worms are completely mature with full oviposition. Thus, it appears that

TABLE 2. Number of *S. mansoni* recovered from 60-day infected mice which rejected the graft

Days after skin grafting	Number of mice	Number of worms		Worm burden	Mean of worm burden
		♀	♂		
10	3	8	11	19	6·3
11	1	8	7	15	15·0
14	2	18	23	41	20·5
16	1	16	16	32	32·0
18	1	17	16	33	33·0

CMI depression depends on an active infection with mature, egg producing worms. It may be determined by substances produced by the adult worms or by the eggs since soluble antigens secreted by the eggs have been implicated in the spontaneous modulation of granulomatous hypersensitivity which has been described in schistosomiasis of long duration (Boros *et al.*, 1975). The rejection of grafts and the formation of granulomas are mediated by cells of the immune system. Of these cells the lymphocytes are known to participate actively in both processes (Wilson & Billingham, 1967). Pelley, Ruffier & Warren (1976) have studied the response of lymphocytes of mice infected with *S. mansoni* to the mitogens Concanavalin A and phytohaemagglutinin. Their data show that 7 weeks after the initiation of egg production (12 weeks of infection) there is a profound unresponsiveness of spleen and lymph-node cells to all concentrations of the mitogens employed. They suggest that suppressor T-cell activity is the most likely explanation for the depression of mitogen reactivity that develops in chronic schistosomiasis. In humans CMI depression may result in persistent septicaemic salmonellosis which occurs quite frequently in chronic schistosoma infection of man (Neves & Lobo Martins, 1967). Further work to determine the effect of specific treatment in mice as well as the degree of CMI impairment in humans infected with *S. mansoni* is in progress.

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REFERENCES

ANDRADE, Z.A. & WARREN, K.S. (1964) Mild prolonged schistosomiasis in mice: alterations in host response with time and the development of portal fibrosis. *Trans. roy. Soc. trop. Med. Hyg.* **58**, 53.

BOROS, D.L., PELLEY, R.P. & WARREN, K.S. (1975) Spontaneous modulation of granulomatous hypersensitivity in Schistosomiasis mansoni. *J. Immunol.* **114**, 1437.

COLLEY, D.G. (1975) Immune responses to a soluble schistosomal egg antigen preparation during chronic primary infection with *Schistosoma mansoni*. *J. Immunol.* **115**, 150.

DOMINGO, E.O. & WARREN, K.S. (1969) Endogenous desensitization: changing host granulomatous response to schistosome eggs at different stages of infection with *Schistosoma mansoni*. *Amer. J. Path.* **52**, 369.

NEVES, J. & LOBO MARTINS, N.R.L. (1967) Long duration of septicaemic salmonellosis: 35 cases with 12 implicated species of *Salmonella*. *Trans. roy Soc. trop. Med. Hyg.* **61**, 541.

PELLEGRINO, J. & SIQUEIRA, A.F. (1956) Técnica de perfusão para colheita de *Schistosoma mansoni* em cobaias experimentalmente infestadas. *Rev. Bras. Malariol. Doenc. Trop.* **8**, 589.

PELLEY, R.P., RUFFIER, J.J. & WARREN, K.S. (1976) Suppressive effect of a chronic helminth infection, Schistosomiasis mansoni, on the *in vitro* response of spleen and lymph-node cells to the T cell mitogens phytohemagglutinin and concanavalin A. *Infect. Immunol.* **13**, 1176.

WARREN, K.S. (1966) The pathogenesis of clay-pipe stem cirrhosis in mice with chronic schistosomiasis mansoni with a note on the longevity of the schistosomes. *Amer. J. Path.* **49**, 477.

WILSON, D.B. & BILLINGHAM, R.E. (1967) Lymphocytes and transplantation immunity. *Advanc. Immunol.* **7**, 189.