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IMMUNODEPRESSION IN MICE FOLLOWING *SCHISTOSOMA MANSONI* INFECTION

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SUMMARY

An immunodepressive state as evaluated by the capacity of the spleen lymphocytes to form rosettes (RFC) and plaque-forming cells (PFC) was observed in mice infected with *Schistosoma mansoni* upon immunization with sheep red blood cells. Animals with a worm load of about 25/per mouse developed a transient immunodepression within the 4th week of infection which lasted about 10 days and thereafter progressively disappeared. Heavily infected animals (50 worms per mouse) did not recover from the depression during the period of observation. Immunodepression was not observed in lightly infected mice.

INTRODUCTION

There has been in the last few years an increasing interest in the effects of *Schistosoma mansoni* infection on the host immunologic response (COLLEY, 1974). It is known that specific serum antibodies to schistosome antigens in mice infected with *S. mansoni* are detectable only several weeks after exposure to cercariae. Antibodies against cercarial or adult worm antigens, when tested by the double-diffusion technique, begin to be found by the 6th to the 7th week of infection (HILLYER & FRICK [5]).

Such a delayed antibody production to schistosomes may be explained by assuming that this parasite yields antigens with low immunogenic capacity (HILLYER & FRICK [5]). However, the immunological capacity of antibody producing cells has not yet been assessed in *S. mansoni* infected mice.

The present investigation was undertaken in order to compare the immunological response of mice with heavy and light *S. mansoni* infections to a thymus-dependent antigen.

MATERIALS AND METHODS

Swiss albino mice (25-35 g) were either exposed to freshly-emerged *S. mansoni* cercariae (L.E. strain, Belo Horizonte, Brazil) by tail immersion or infected by subcutaneous injection. At various times, as stated in each experiment, a group of six infected mice were intravenously injected with 0.3 ml of a washed sheep red blood cells (SRBC) at 9×10^8 cell suspension. Four or six days later the animals were killed, the abdomen opened and the spleen removed and weighed. One half of the spleen was used to perform

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the rosette-forming cells (RFC) test and the other half assayed in the plaque-forming cell test (PFC). Each half spleen was disrupted gently in a glass homogenizer in Ringer-Tris solution pH 7.4, and the cells washed three times at 4 C by resuspending them with the same solution and centrifuging for 7 min at 100 g. The cells for RFC test were resuspended in the above solution and adjusted to 2×10^7 cells/ml. The cells for PFC test were resuspended in 2 ml of TC-MEM (minimal Eagle medium-Hank's BSS, Difco) containing 10% fetal calf serum. When only PFC test was to be performed, the cells of the whole spleen were resuspended in 4 ml of the above solution. Controls were run with spleens from normal mice.

The technique used to assess rosette formation (BIOZZI et al. [1]) consisted in mixing 0.3 ml of 6×10^6 nucleated cells with 0.25 ml of SRBC suspension at 1×10^8 cells/ml, plus 0.4 ml of Ringer-Tris solution pH 7.4 and 0.05 ml of normal mouse serum previously adsorbed three times with SRBC. This mixture was allowed to stand overnight at 4°C. The agglutination of at least 4 erythrocytes on a nucleated cell was defined as a rosette. The number of rosettes was counted in a hemocytometer and the results were expressed either as RFC: 1000 spleen cells or RFC: spleen.

Antibody-forming cells were assayed by a slight modification of the method of hemolytic plaque-forming cells (PFC) described by CUNNINGHAM & SZENBERG [4]. Briefly, 3 pieces of "double-sided" tape (Scotch-brand 410) were laid across a microscope slide dividing it into two equal areas. Two coverlips were then pressed firmly on the tape to form two shallow chambers. A mixture made at room temperature of the spleen cell suspension, fresh guinea pig serum (1:10) as a source of complement, and SRBC (2×10^8 cells/ml) was applied to the side of the chambers until the narrow space between the slide and coverlips became filled. After sealing the borders of the slides with heated paraffin-vaseline (1:1) mixture, they were incubated at 37°C for 30 min and the hemolytic plaques were counted at low magnification. The results were expressed either as PFC: 1000 spleen cells or PFC: spleen. In the experiment depicted in Fig. 1 the hepatic and mesenteric veins of infected animals were perfused to assess the worm burden.

For statistical analysis of the data, results from groups of animals in each experiment were compared with appropriate controls. The Student's test was used to determine levels of significance.

RESULTS

Table I shows that the proportion of RFC in spleen cells of mice exposed to *S. mansoni* cercariae decreases significantly in the period from day 28 to day 31 of infection. PFC paralleled the depression observed in the RFC test with significant reduction around the same days after infection (Table II). Although a slight depression persisted after the 34th day, comparison with the control group showed that the difference was not significant.

In other experiments, known numbers of cercariae (Fig. 1 A, B, C) were used to infect mice. At the infection times indicated in Fig. 1, the spleens of infected and control animals were weighed before homogenization for PFC determinations. Simultaneously, perfusions were carried out to estimate the worm burden of the animals. Figure 1 A clearly shows that the immunodepression does not occur in animals with light infection. Because of the small size of the worms during the first 4 weeks of infection it was difficult to determine accurately the worm load at this period. Coincident with the decrease in spleen weight the immunodepression became apparent ($p < 0.05$ at the days 30, 33, 37 — Fig. 1 B and C). In spite of the hyperplasia observed in spleens after the 37th day of infection, the antibody response to SRBC was still partially suppressed in the groups of mice which were heavily infected (Fig. 1 C) and persisted during the period of observation. Three out of six mice from this group died at the 49th day of infection. On the other hand the group which yielded an intermediate worm load showed a greater increase in the spleen weight and the PFC returned to normal levels.

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TABLE I

The ability of spleen cells from mice infected with *S. mansoni* cercariae to form rosettes after immunization with SRBC

Days after infection (*)	RFC per 10^3 spleen cells		p	% of inhibition
	Control	Infected		
7	5.3 ± 2.6	8.3 ± 2.5	< 0.50	—
12	5.2 ± 1.1	4.8 ± 1.5	< 0.50	6.4
17	4.1 ± 2.4	3.9 ± 1.5	< 0.50	6.3
21	5.3 ± 1.2	6.3 ± 3.0	< 0.50	—
25	8.4 ± 3.2	5.6 ± 0.8	< 0.10	33.1
27	6.3 ± 2.7	4.0 ± 1.1	< 0.10	35.7
28	7.8 ± 1.2	4.4 ± 1.7	< 0.005	43.4
30	3.6 ± 1.0	1.0 ± 0.4	< 0.005	73.0
31	5.4 ± 2.0	2.4 ± 0.8	< 0.025	55.0
34	5.4 ± 1.7	3.6 ± 1.9	< 0.25	33.6
37	5.1 ± 1.5	4.5 ± 2.2	< 0.50	15.7
40	4.6 ± 1.6	3.6 ± 1.3	< 0.50	21.6
51	6.5 ± 4.2	4.5 ± 1.2	< 0.50	30.8
59	5.2 ± 1.8	4.0 ± 1.1	< 0.25	22.1

(*) Mice infected on day one with 200 *S. mansoni* cercariae
Animals per group: 5 to 7

TABLE II

The ability of spleen cells from mice infected with *S. mansoni* cercariae to form hemolytic plaques after immunization with SRBC

Days after infection (*)	PFC per 10^3 spleen cells		p	% of inhibition
	Control ± SD	Infected ± SD		
7	2.0 ± 0.7	2.2 ± 0.9	< 0.50	—
12	2.4 ± 0.4	2.7 ± 0.8	< 0.50	—
17	1.2 ± 0.2	1.4 ± 0.9	< 0.50	—
21	2.1 ± 0.9	1.7 ± 0.7	< 0.50	17.7
25	2.4 ± 0.6	1.6 ± 1.0	< 0.25	32.4
27	2.0 ± 1.1	0.7 ± 0.5	< 0.05	63.1
28	3.3 ± 1.3	0.7 ± 0.1	< 0.05	80.1
31	2.0 ± 1.3	0.7 ± 0.3	< 0.05	63.3
34	1.1 ± 0.5	0.7 ± 0.4	< 0.25	40.2
37	0.9 ± 0.2	0.9 ± 0.3	< 0.50	—
40	0.8 ± 0.4	0.5 ± 0.4	< 0.25	38.3
51	2.2 ± 0.5	1.9 ± 0.3	< 0.25	14.7
59	2.4 ± 0.6	1.6 ± 1.0	< 0.25	32.4

(*) Mice infected on day one with 200 cercariae of *S. mansoni*
Animals per group: 5 to 7

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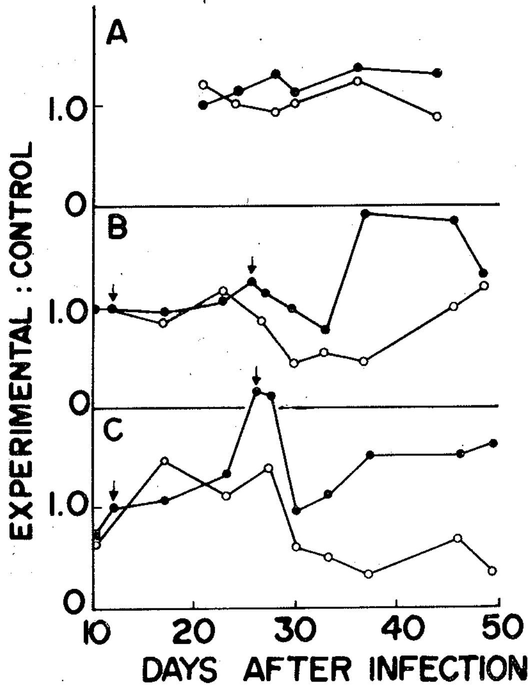

Fig. 1 — Influence of *S. mansoni* infection on the PFC response of mice to an intravenous injection of 3×10^8 sheep red blood cells (SRBC). Data expressed as the mean ratio of spleen weight (●——●) or number of PFC per spleen (o——o) of experimental to control mice (e/c). The average number of worms recovered per mouse in experiments A, B, and C was respectively 15, 25, and 50. Arrows indicate spleens weight of animals non injected with SRBC.

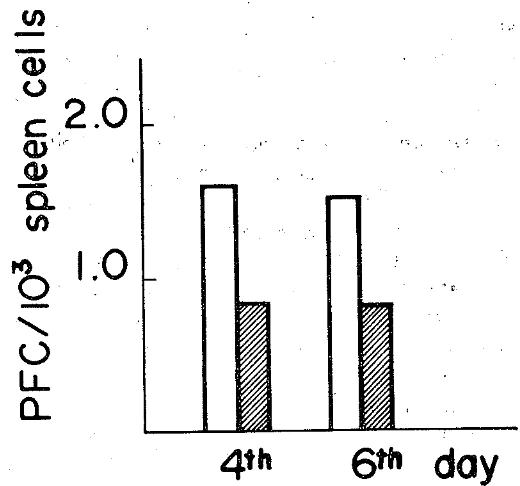

Fig. 2 — Response of mice on the 30th day of infection to SRBC injected on the 4th and 6th day prior to PFC determination. Dashed and open bars indicate the mean of PFC per 10^3 spleen cells of 6 infected or control mice, respectively.

In order to examine the possibility that *S. mansoni* infection could have merely altered the time course of the immune response, PFC tests were carried out at the 4th and the 6th day after the SRBC stimulus. Figure 2 allows this hypothesis to be discarded for the response was practically the same if performed at the 4th or the 6th day.

DISCUSSION

These experiments demonstrate that during the first three weeks of an *S. mansoni* infection mice do not differ significantly in their antibody responsiveness to a SRBC stimulus from the control group. However, within the 4th week the animals with a worm load of about 25 worms per mouse (Fig. 1 B) developed a transient immunodepression which lasted about 10 days. The group of heavily infected animals (Ca. 50 worms/mouse — Fig. 1 C) did not recover from the depression during the period of observation. The data obtained by the different techniques employed to evaluate the immune status of the animals were coincident with the highest percentage of the adult schistosomes in the liver and mesenteric vessels (WILKS [7]). An increase in the spleen weight and number of cells occurred just before the reduced response to SRBC was observed, and it was more pronounced in the group of mice infected with higher worm load (Fig. 1 C). In some cases the total spleen cell counts were twice that of the controls. This hyperplasia was not due to the SRBC stimulus since it was also observed in non-stimulated controls (Fig. 1 B and C). Another peak of hyperplasia occurred within the 6th week after infection which lasted until the experiments were interrupted. This period coincides with the beginning of the oviposition and the initiation of anti-egg immune responses (COLLEY [2, 3]).

The immunodepressive state observed could be explained by an immunodepression of T lymphocyte function at high levels of worm-

related antigens. Alternatively one could hypothesize the production of substance(s) with immunosuppressive activity by *S. mansoni* at that particular stage of its development.

The immunodepression is apparently non-specific since it was detected by using sheep red blood cells, whose antigenic composition differ considerably from that of *S. mansoni*. Due to the T lymphocyte-dependent nature of the anti-SRBC response it is impossible to relate the immunosupressive activity to T or B lymphocytes or say if some other cell type is involved. In addition some protozoal infections are potentiated by *S. mansoni* infections. It is well-known, for instance, that schistosomiasis modifies the course of *Plasmodium berghei* infection in *Microtus guenteri* (YOELI [8]) and affects severely the parasitemia in mice infected with *T. cruzi* (KLOETZEL et al. [6]).

The possible role of the immunodepression here reported in determining the acute form of schistosomiasis has been recently suggested by COLLEY [3].

RESUMO

Imunodepressão em camundongos infectados pelo Schistosoma mansoni

Estado imunodepressivo, avaliado pela capacidade de linfócitos de baço de formar rosetas (RFC) e placas (PFC) foi observado em camundongos infectados com *Schistosoma mansoni* e sensibilizados com hemácias de carneiro (SRBC). A imunodepressão aparece na 4.ª semana e é transitória com uma carga parasitária de 25 vermes por camundongo. Animais infectados com 50 vermes não se recuperaram de imunodepressão no período de observação. Não se verificou o fenômeno em animais infectados com um número de vermes inferior a dez.

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