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EVALUATION OF SPLEEN CELL POPULATION AND EFFECT OF SPLENECTOMY ON GRANULOMA MODULATION IN BALB/C MICE INFECTED WITH *SCHISTOSOMA MANSONI*

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A kinetic study of the cells present in the spleen of BALB/c mice infected with Schistosoma mansoni *was carried out. The lymphocytes were evaluated phenotypically with monoclonal antibodies and the effect of splenectomy on the modulation of periovular granuloma was also investigated. The infected mice had proportional increases in the numbers of neutrophils, plasma cells, macrophages and eosinophils in the spleen. The largest number of neutrophil, plasma cells and macrophage were found between the 8th and the 12th week of infection, while the amount of eosinophils were higher later on, around the 20th week. The lymphocytes phenotipically characterized as Thy 1.2, Lyt 1.2 (CD4) increased mildly in proportional numbers. However, the percentage of lymphocytes with the Lyt 2.2 (CD8) phenotype, which is characteristic of supressor and cytotoxic T cells, increased significantly with the progress of the disease. The numbers of B lymphocytes, which comprise 50% of the mononuclear cells present in the spleen, increased significantly till the 16th week when they began to decrease. The mean diameters of periovular granulomas were comparatively similar in both experimental groups (splenectomized and non-splenectomized mice). However, the evolutive types of granuloma (exudative, intermediate and fibrous) in splenectomized mice were proportionally different from those of non splenectomized mice in the 16th and 24th week of infection. It is inferred that lymphonodes or other secondary lymphoide organs, in the absence of the spleen, assume a modulating action on periovular granulomas, although the evolution of the granulomas is somehow delayed in splenectomized mice.*

Key word: *Schistosoma mansoni* – murine schistosomiasis – granuloma modulation – splenectomy – lymphocytes

In murine schistosimiasis mansoni the granulomatous response evoked by schistosome eggs is a major factor in the pathogenesis of the disease and is largely attributable to a cell-mediated response to soluble egg antigen (SEA) (Boros & Warren, 1970; Warren, 1972). The importance of T-cell-mediated delayed-type-hypersensitivity in the process of granuloma formation has been demonstrated experimentally in athymic (Byram & Von Lichtenberg, 1977) and thymectomized animals (Domingo & Warren, 1967). The intensity of the response is maximum at eight weeks of infection and declines to a minimum by 30 weeks (Andrade & Warren, 1964). This phenomenon has been termed spontaneous modulation. In the acute phase, the granulomatous response is rich in neutrophils and eosinophils with few mononuclear cells while, in the late phase, there are fibrous tissue and few acute inflammatory cells.

Colley (1976) has reported that hepatic granuloma formation decreased in infected mice following adoptive transfer of splenic mononuclear cells from chronically-infected animals. Administration of T lymphocytes from acutely and chronically infected donors resulted in significant suppression of granulomas. Treatments with allogenic antigen and complement, that selectively eliminated certain subsets of lymphocytes showed that the enhanced granulomatous response is Lyt 1 cell-dependent. In contrast, the lymphocytes from chronically infected mice were classified as Lyt 2,3+, T supressor cells. In fact, when chronically

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infected mice were treated with cyclophosphamide, a drug known to kill Ts cells, no modulation of the granulomatous response was observed (Colley et al., 1979; Chensue et al., 1981). Finally, when eight weeks infected mice were splenectomized, a further enhancement of the granulomatous response was noticed (Hood & Boros, 1980). The splenectomy possibly eliminated the Ts cells and abrogated the granulomatous modulation.

The purpose of this study was to evaluate the kynetics of splenics cells in *Schistosoma mansoni*-infected mice. T lymphocytes were evaluated with monoclonal antibodies and the effect of previous splenectomy on the hepatic granulomas was also investigated.

MATERIALS AND METHODS

Infection and splenectomy – BALB/c mice were obtained from FIOCRUZ in Rio de Janeiro and infected transcutaneously with 50 cercariae of *S. mansoni* (Feira de Santana-BA strain). The animals were divided into three experimental groups: I – infected mice II – splenectomized infected mice, and III – control mice. Group II was splenectomized one week prior to infection, under pentobarbital anesthesia. The skin was shaved, disinfected, and incised with sterile instruments. The peritoneum was open, and the spleen removed. The abdomen was carefully inspected for splenic remnants. The peritoneum and skin were then sutured. The mice of group I were killed at 4, 8, 12, 16, 20 and 24 weeks of infection. Mice in group II were killed at 6, 12, 16 and 24 weeks. Spleens and livers were weighed, sliced, and fixed in buffered formalin (pH = 7.2) for histological purposes. Sections were cut at 3-5 μm and stained with hematoxilin and eosin.

Size and types of granulomas – Granuloma diameter was measured in two axes with an ocular micrometer (OSM-Olympus) adapted to the microscope. Granulomatous reactions were classified into three categories: I – exudative type, II – intermediate type, and III – fibrous type, as previously described (Reis & Andrade, 1987).

Cell determinations – Each spleen was homogenized in a Potter Elvehjem tissue homogenizer with RPMI 1640 (Gibco, Grand Island, N.Y.). Cell smears were obtained by using a cytocentrifuge (Cytospin-Shandon Southern, U.S.A.). The spleen cell differential count was determined by counting 100 cells stained with Wright-Giemsa.

Mononuclear cell separation and T enriched lymphocytes – Mononuclear cells were obtained using a mononuclear gradient centrifugation medium (Lympholyte-M, Cedarlane, Ontario, Canada). T and B lymphocytes were separated, using a nylon-wool column as described (Julius et al., 1973). The percent contaminating B cells in the pool of T lymphocytes was determined by immunofluorescent staining with polyclonal rabbit anti-mouse Igs FITC conjugate (Sigma Chemical Company, St Louis, U.S.A.). The T lymphocyte subsets were classified by their membrane antigens with anti-Thy 1.2 (1:250 dilution), anti-Lyt 1.2 (CD4) (1:250 dilution), and anti-Lyt 2.2 (CD8) (1:40 dilution) monoclonal antibodies (Cedarlane Laboratories, Canada) using immunofluorescence technique. The second antibody was FITC/conjugated rabbit anti-mouse IgG and IgM (1:20 dilution).

The proportional numbers were calculated by counting 100 cells/spleen in a epifluorescence microscope (American Optical vertical Fluorescence model 2071, U.S.A.).

Statistical analysis – Spleen cell data were analyzed with the non-parametric Mann-Whitney test. The granuloma diameters were compared with a Chi-squared test. For comparing spleen and liver weights of infected animals and controls, the student's T test was used. Results were considered significant at the $P < 0,05$ level.

RESULTS

The spleens of four weeks infected mice were significantly heavier than those of control mice (Table I). At six weeks, the spleen of infected animals weighed three times more than those of the control animals (0.376 g/0.1g) and at 16 weeks four times more (0.47 g/0.1g). Liver weights were similar for both splenectomized and non-splenectomized infected mice and significantly higher than those of the control mice (Table II).

The effect of splenectomy on hepatic granuloma diameter is shown in Table III. During *S. mansoni* infection the granulomatous reaction around schistosome eggs decreased in both splenectomized and non-splenectomized infected mice, with similar granuloma diameters noted in both groups.

TABLE I

Variation of spleen weights during *Schistosoma mansoni* infection in Balb/c mice

Weeks of infections	Experimental groups (mean ± SD)			
	n	Infected	n	Controls
4	8	0.15 ± 0.03	3	0.12 ± 0.01
8	5	0.37 ± 0.11	4	0.10 ± 0.01[a]
12	6	0.37 ± 0.10	4	0.12 ± 0.20[a]
16	5	0.47 ± 0.13	4	0.11 ± 0.01[a]
20	7	0.44 ± 0.09	4	0.11 ± 0.01[a]
24	6	0.43 ± 0.15	6	0.10 ± 0.02[a]

Weight: given in grams; n: mice in each group.
a: significant $p < 0.05$ (student's test).

TABLE II

Variation of liver weights in splenectomized and non-splenectomized and non-splenectomized infected and control mice

Weeks of infection	Experimental groups (mean ± SD)					
	n	Infected non splenectomized	n	Infected splenectomized	n	Controls
8	5	1.39 ± 0.1	5	1.46 ± 0.4	4	0.97 ± 0.03
12	6	1.5 ± 0.1	6	1.55 ± 0.1	6	1.12 ± 0.1[a]
16	5	1.4 ± 0.1	6	1.47 ± 0.1	4	1.04 ± 0.1[a]
24	6	1.3 ± 0.3	6	1.27 ± 0.2	6	1.08 ± 0.06[a]

Weight: given in grams; n: mice in each group.
a: significant when compared to infected by student's test.

TABLE III

Comparison of granuloma diameter in splenectomized and non-splenectomized Balb/c mice

Weeks of infection	Experimental groups (mean ± SD in micrometers)			
	G	Non splenectomized	G	splenectomized
8	100	408.6 ± 64.4	99	410.2 ± 58.1
12	99	382.0 ± 57.0	97	403.5 ± 43.9
16	68	269.0 ± 56.2	119	275.8 ± 45.9
24	128	220.5 ± 62.0	105	225.1 ± 38.4

G: number of granulomas evaluated.

The inflammatory reactions surrounding schistosome eggs were classified into three groups. During the first 12 weeks of infection, both groups of animals showed the same proportion of granuloma types. However, after the 12th week, granuloma of the intermediate type was more common in the splenectomized mice than in non-splenectomized animals (52% vs 25% at 24 weeks), while the splenectomized animals have shown fewer type I and II granulomas than non-splenectomized mice.

TABLE IV

Types of cells in spleens of *Schistosoma mansoni* infected Balb/c mice

Cell type	Weeks of infection	% and range
Lymphocytes	4	84.1 (75.1 – 88.6)
	8	60.5 (49.0 – 74.0)
	12	49.3 (35.4 – 60.3)
	16	71.1 (68.0 – 77.3)
	20	77.1 (65.6 – 84.6)
	24	67.6 (59.6 – 77.8)
Neutrophils	4	9.3 (4.6 – 20.8)
	8	18.6 (11.0 – 28.0)
	12	29.0 (18.0 – 43.6)
	16	15.2 (12.0 – 16.5)
	20	9.2 (4.8 – 18.1)
	24	19.0 (10.5 – 24.0)
Macrophages	4	3.7 (2.0 – 7.3)
	8	6.2 (4.0 – 9.6)
	12	9.8 (7.2 – 12.4)
	16	5.0 (2.4 – 7.0)
	20	4.0 (2.0 – 6.8)
	24	4.5 (2.2 – 7.6)
Eosinophils	4	0.1 (0.0 – 1.0)
	8	1.2 (0.0 – 2.1)
	12	3.7 (0.0 – 6.9)
	16	4.5 (2.0 – 6.7)
	20	5.3 (3.0 – 6.8)
	24	3.8 (1.4 – 7.6)
Plasma cells	4	0.6 (0.0 – 1.3)
	8	3.5 (2.4 – 1.3)
	12	5.9 (4.5 – 7.7)
	16	2.2 (0.7 – 2.4)
	20	1.3 (0.0 – 2.4)
	24	3.1 (0.0 – 8.6)

The cells present in the spleens of *S. mansoni* infected mice are shown in Table IV. Lymphocytes, the predominant splenic cells, correspond to 90% of the cells in the spleens of normal mice. The percentage of lymphocytes in infected animals is less as other cell types increase during infection. The lowest lymphocyte level was found at week 12. Other cell types, including neutrophils, macrophages, eosinophils and plasma cells increased during *S. mansoni* infection. The proportion of neutrophils present in normal spleens was 3 to 7%, while in the spleens of infected animals increased to 9-29%. Eosinophils were present at 0 to 1% of cells in normal spleens and at 5.3% in the spleens of infected animals. Macrophages and plasma cells were frequently found in smears of spleen cells, but not as common as lymphocytes and neutrophils. Macrophages constituted 3.7 to 9.8% of spleen cells,

while plasma cells constituted 0.6 to 5.9%. All cell types except lymphocytes, were higher in infected mice than in control mice.

The kinetic of spleen cells during the 24th week of *S. mansoni* infection in mice indicated that the predominant cells were lymphocytes, followed by neutrophils, macrophages, eosinophils, and plasma cells.

T cell subsets were also evaluated using monoclonal antibodies (anti-Thy 1.2, Lyt 1.2 (CD4) and Lyt 2.2 (CD8) antigens). Infected mice showed more Thy 1.2+ and Lyt 1.2+ lymphocytes as compred with control mice, but without relation to the lenght of *S. mansoni* infection. About 50 to 70% of the T cells obtained by nylon wool-enrichment were Thy 1.2+ or Lyt 1.2+, a higher proportion than that of control animals (Tables V, VI).

TABLE V

Proportion of Thy 1.2 lymphocytes in nylon wool-enriched T cells from the spleens of *Schistosoma mansoni* infected and controls Balb/c mice

Weeks of infection	Experimental groups: Infected (% and range)	Experimental groups: Controls (% and range)
4	61.0 (57.6 – 66.3)	48.2 (43.6 – 53.0)[a]
8	54.8 (51.4 – 58.0)	48.7 (47.5 – 50.0)[a]
12	60.9 (58.0 – 62.5)	62.4 (54.9 – 70.0)
16	61.6 (52.0 – 68.0)	50.5 (49.0 – 52.0)
20	55 (40.0 – 67.5)	38.3 (30.0 – 46.6)
24	65.8 (57.7 – 69.3)	43.0 (38.0 – 46.6)

a: statistically significant ($p < 0.05$) by Mann Whitney test.

TABLE VI

Proportion of Lyt 1.2 (CD4) lymphocytes in nylon wool-enriched T cells from the spleens of *Schistosoma mansoni* infected and controls Balb/c mice

Weeks of infection	Experimental groups: Infected (% and range)	Experimental groups: Controls (% and range)
4	65.6 (60.0 – 70.3)	52.4 (47.6 – 57.4)[a]
8	48.4 (42.5 – 57.2)	50.7 (49.0 – 52.4)
12	58.9 (35.7 – 72.2)	51.4 (49.0 – 54.0)
16	70.4 (63.0 – 82.0)	49.0 (40.0 – 58.0)[a]
20	66.3 (63.3 – 73.7)	47.5 (47.5 – 54.0)[a]
24	64.5 (57.0 – 68.3)	51.1 (47.0 – 59.2)

a: statistically significant ($p < 0.05$) by Mann Whitney test.

The proportion of suppressor/cytotoxic lymphocytes found in the spleens of *S. mansoni* infected mice increased as early as the 12th week of infection. As shown in Table VII, in the early acute phase (4th to 8th weeks of infection), the Lyt 2.2 (CD8) levels were similar to those of control mice. However, after the 12th week, the Lyt 2.2 (CD8) proportion in nylon wool-enriched T cells increased from 8.1% to 20.8% by the 24th week.

TABLE VII

Proportion of Lyt 2.2 (CD8) lymphocytes in nylon wool-enriched T cells from the spleens of *Schistosoma mansoni* infected and controls Balb/c and control mice

Weeks of infection	Experimental groups: Infected (% and range)	Experimental groups: Controls (% and range)
4	5.9 (5.0 – 8.0)	4.9 (2.9 – 7.0)
8	5.4 (4.0 – 6.0)	6.0 (5.0 – 7.0)
12	8.1 (4.0 – 15.0)	5.4 (4.9 – 6.0)
16	6.5 (4.9 – 8.0)	2.4 (1.9 – 3.0)[a]
20	12.5 (5.6 – 18.5)	7.3 (6.7 – 8.0)
24	20.8 (14.4 – 25.7)	8.0 (7.0 – 9.2)[a]

a: statistically significant ($p < 0.05$) by Mann Whitney test.

The amont of B lymphocytes (data nor shown) was higher in infected than in non infected animals, except during the 24th week. The proportion of spleen mononuclear cells that were B lymphocytes ranged from 43% to 58%.

DISCUSSION

Following *S. mansoni* infection of BALB/c mice and the beginning of oviposition, circulating SEA (soluble egg antigens) stimulates lymphoid spleen cells, thus causing increased splenic cellularity and weight (splenomegaly). The largest spleens were observed at the 16th week of infection. Throught the course of this study, infected mice had significantly larger spleens than the control mice. Andrade (1962) and Andrade & Andrade (1965) described this hypercellularity of the white pulp and venous congestion of the spleen in experimental and human schistosomiasis.

Evaluation of the cell types showed the spleens of infected mice have more lympho-

cytes, neutrophils, eosinophils, macrophages, and plasma cells than those of normal animals. Histological results were similar, showing acute splenitis secondary to infection. Neutrophils were more frequently seen than eosinophils, plasma cells, and macrophages. It has been reported that neutrophil precursors are increased in mouse bone marrow and decreased in peripheral blood (Santos da Silva et al., 1988). Other histopathological studies have shown more eosinophils than neutrophils (Andrade & Andrade, 1965). In the present kinetic study of spleen cells, we have noticed that in the 12th week of infection, the numbers of neutrophils, macrophages, and plasma cells are highest, while the numbers of eosinophils peak during the 20th week.

The proportion of B lymphocytes observed by us varied between 35.9% to 58% of mononuclear cells and decreased after the 20th week. This result was similar to that of Chensue & Boros (1979a).

Absence of the spleen affected the modulation of hepatic granulomas only in the late phases of the infection. The major difference between granuloma types was in the amount of fibrous tissue. Thus, the spleen may play a role in egg granuloma fibrogenesis. Hood & Boros (1980) showed higher levels of hepatic collagen in splenectomized mice than in non-splenectomized ones. Splenectomy in the 8th week of infection affected granuloma modulation. The granuloma diameters in splenectomized animals were greater than in controls and non-splenectomized animals. However, when surgery was performed between the 1st and the 4th week of infection, there was no effect on granuloma size.

The circulation of lymphocytes in the spleen and other lymphoid organs (Rocha et al., 1983) may explain why splenectomized animals modulate the inflammatory reaction around the schistosome eggs in absence of the spleen prior to the 8th week following *S. mansoni* infection. It has been observed that transfer of spleen cells from chronically infected mice to acutely infected animals decrease the granulomatous response. Treatment with the monoclonal antibody anti-Thy 1.2 abrogated this modulation (Chensue & Boros 1979b). Our results showed that as the murine schistosomiasis progresses, the population of Thy 1.2 Lyt 2.2 supressor lymphocytes is significantly increased.

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