

Malnutrition and Hepatic Fibrosis in Murine Schistosomiasis

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In this paper, four different approaches attempting to reproduce the schistosomal liver fibrosis in undernourished mice are reported: shifting from a deficient to a balanced diet and vice-versa, repeated infections, influence of the genetic background, and immunological response. Infections were performed with 30 cercariae of Schistosoma mansoni and lasted at least four months. Undernourished mice were unable to reproduce the picture of "pipestem" fibrosis, except the C57 BL/10 inbred strain, four out of 21 mice developing the liver lesion. A link of this histological finding to the type of parasite strain can not be discarded at the moment. Repeated infections increased collagen deposition mainly in well nourished animals (seven out of 16 Swiss mice developed "pipestem"-like fibrosis). In undernourished infected Swiss mice the serum levels of soluble egg antigen specific antibodies IgG1, IgG2a, IgG2b, and IgG3 were two to four times lower than those detected for well nourished controls. The decreased humoral immune response coupled to the morphological, morphometric, and biochemical results reinforce the influence of the host nutritional status on the connective tissue changes of hepatic schistosomiasis.

Key words: *Schistosoma mansoni* - undernutrition - hepatic fibrosis - mouse

Schistosomal hepatic fibrosis (Symmers' fibrosis) is a typical primary fibrosis involving essentially portal spaces, whose aetiopathogenesis is still incompletely understood. In humans, both periportal granulomatous lesions, and the diffuse progressive periportal fibrosis participate in the formation of fibrotic tissue (Andrade 1965, 1987b, Andrade & Bina 1983, Grimaud & Borojevic 1986).

The elucidation of the pathophysiology and pathogenesis of a disease almost invariably requires the development of an animal model. Concerning Symmers' fibrosis (clay pipestem fibrosis), different experimental hosts have been tried (Cheever et al. 2003), such as monkeys (Lichtemberg & Sadun 1968, Sadun et al. 1970, Lichtemberg et al. 1971, Damian et al. 1976, Farah et al. 2000), rabbits (Cheever et al. 1980), pigs (Hurst et al. 2000), and mice (Warren & DeWitt 1958, Andrade & Warren 1964, Cheever 1965, Warren 1968, Andrade 1987a, Andrade & Cheever 1993, Andrade et al. 1997). In this last animal model, during mild (one to two pairs of worms) and prolonged (16 weeks or more) infections with *Schistosoma mansoni*, a picture mimicking "clay pipestem" fibrosis seen in humans with advanced schistosomiasis has been reproduced in outbred and in some strains of well-nourished inbred mice (Andrade & Cheever 1993), but not in undernourished outbred specimens (Coutinho et al. 1997).

The importance of an adequate nutritional status for a healthy condition of the host is well recognized. According to 1990 figures from the Disease Control Priorities Project in Developing Countries (Mason et al. 2003), it is estimated that 32% of the global burden of disease (mortality and morbidity) in these countries would be removed

by eliminating malnutrition. In endemic zones for *S. mansoni* infection, an overlapping of undernutrition and parasite infection is frequently observed. However, incomplete and sometimes conflicting results have been reported in the literature on the role of the host nutritional status as a probable co-factor in the pathogenesis of advanced clinical forms of schistosomiasis.

Since time and parasite load are not sufficient to explain all the cases, it was decided to study the liver pathology in the undernourished murine model under different experimental situations. It is known, based upon parasitologic, histopathologic, biochemical, and morphometric data, that undernourished mice develop smaller circumoval granulomas, less intense portal inflammation, and minimal liver fibrosis, when compared with control animals. On the other side, undernourished mice are unable to develop the "pipestem" - like portal lesion (Coutinho et al. 1997) as approximately 30-50% of well-nourished controls usually do (Andrade & Cheever 1993).

The purpose of this paper is to report on four different approaches that are being tried in our laboratory, in successive attempts to reproduce and explain the pathogenesis of the "pipestem" - like schistosomal hepatic fibrosis in undernourished mice.

MATERIALS AND METHODS

Animals - Male albino Swiss mice (21 day-old), weighing 11 to 15 g (Experiments I, II, IV) and inbred female mice (BALB/c and C57BL/10 strains), from the Animals Breeding Center-Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, Brazil (Experiment III) were used. Animals were kept in individual wire bottom cages. Water and food were provided ad libitum. All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of the Fiocruz.

Infection - Each mouse was exposed to 30 cercariae of *S. mansoni* (percutaneous route) shed from laboratory raised and infected *B. glabrata*. The BH strain (Belo Horizonte, MG, Brazil) was used in Experiments I, II, IV;

and the SLM strain (São Lourenço da Mata, PE, Brazil) in Experiment III (see Experimental groups). Infections lasted 24 weeks (Experiment I), 20 weeks (Experiment III), and 16 weeks (Experiment IV). In Experiment II, reinfections were performed starting 45 days after the primary infection (5 reinfections with 15 cercariae each per mouse, at 15 day intervals). In all the experiments, mice were first infected after a period of four weeks ingesting their respective diet.

Diets - Undernutrition was induced by feeding mice with a multideficient and essentially low-protein diet (7-8% protein), planned to simulate that usually ingested by low-income individuals living in endemic areas of Manson's schistosomiasis in Northeast Brazil (Coutinho et al. 1997) and is thus referred to as regional basic diet (RBD). Control diet (NUVILAB) was a pelleted commercial balanced chow for mice produced by Nuvital Nutrients Ltda. (Colombo, PR, Brazil), with 22% protein content.

Evaluation of nutritional status - Body weight weekly recorded and food consumption measured every day were the parameters used to evaluate the nutritional status of the mice. The experimental model used has extensively been studied in previous investigations (Coutinho 1980, Teodósio et al. 1990, Coutinho et al. 1992, 1997).

Experimental groups

Experiment I - Shifting from a deficient to a balanced diet and vice-versa: Group G1, "early undernutrition" - RBD was offered during 16 weeks and then replaced by NUVILAB for a further 8 weeks period; Group G2, "late undernutrition" - Mice were maintained on NUVILAB for 16 weeks, and then shifted to RBD for 8 weeks; Group CG3, "long-lasting undernutrition" (Controls for group G1) - The RBD-diet was offered throughout the whole experiment; Group CG4, "long-lasting" normal feeding (Controls for group G2) - Mice were maintained on NUVILAB throughout the experiment.

Results concerning Experiment I were fully reported in *Mem Inst Oswaldo Cruz* 98(7): 919-925, 2003 and are only summarized in this paper.

Experiment II - Repeated infections: Group A1, undernutrition, single infection; Group A2, normal feeding, single infection; Group B1, undernutrition, repeated infections; Group B2, normal feeding, repeated infections.

Experiment III - Host genetic background: Group A1, undernourished BALB/c mice; Group B1, well-nourished BALB/c mice; Group A2, undernourished C57BL/10 mice; Group B2, well-nourished C57BL/10 mice; Group C1, undernourished outbred Swiss mice (controls); Group C2, well-nourished outbred Swiss mice (controls).

Experiment IV - Immunological response: Group A, undernourished, non-infected; Group B, undernourished, infected; Group C, well-nourished, non-infected; Group D, well-nourished, infected.

Morphological studies - Mice were sacrificed by cervical dislocation. The livers were removed, rinsed with PBS (phosphate buffered saline), weighed, and divided into several portions. One section was placed in Bouin's fixative and/or in buffered (pH 7.4) 10% formaldehyde for histologic examination. Tissue was embedded in paraffin

and the 5 μ m thick sections obtained were stained with haematoxylin-eosin and picosirius red method for collagen (Junqueira et al. 1979). A portion of the liver was placed in 4% potassium hydroxide for egg counting (Cheever 1970). Another sample was frozen at -70°C for further biochemical quantification of hydroxyproline.

Morphometry - Randomly sampled 5 μ m-thick liver histological sections stained with picosirius-red for collagen, were examined by semiautomatic morphometry using the LEICA Qwin 2.6 Image Processing and Analysis System (Leica Cambridge, Cambridge, England) coupled to a LEICA DC 300F digital camera. For morphometric measurements a total sectional area of 6.6 mm^2 per animal was evaluated. All periovular granulomas were included. A spherical shape and normal size distribution were assumed. The following parameters were calculated for granulomas: size, volume density, and numerical density. The sectional area of the fibrous tissue red stained was directly measured and calculated as a percentage of the total area examined, as previously described (Barbosa Jr. 2001, Coutinho et al 2003).

Biochemical study - A sample of the liver was frozen at -70°C for determination of collagen, measured as hydroxyproline by the spectrophotometric method B of Bergman and Loxley (1963). Hydroxyproline levels were corrected for intensity of infection with help of a simplified electronic spread sheet elaborated by Cheever (1986) and used in subsequent papers.

Parasitologic studies - Worms recovered after perfusion of the portal system (Duvall & DeWitt 1967) were counted and separated by sex. Quantification of the number of eggs in liver tissue was performed after digestion (Cheever 1970).

Immunological studies - Preliminary results are based on lymphoproliferative assays using cultures of spleen cells stimulated with mitogen concanavaline A (1 $\mu\text{g}/\text{ml}$) or soluble egg antigen - SEA (10 $\mu\text{g}/\text{ml}$); production of the cytokines IFN- γ , IL-4, IL-5 by cultured spleen cells after stimulation with SEA and con A (ELISA immunoenzymatic assay); and titration of anti-SEA serum antibodies IgG1, IgG2a, IgG2b, IgG3 (ELISA).

Statistical analysis - Data were analyzed by the following methods: Student's "t-test (when appropriate), one-way ANOVA with Tukey's and Tamhane's post tests, Mann-Whitney's and Kruskal-Wallis' non parametric tests (SPSS v.8 - Statistical Package for Social Sciences Incorporation, US) and EXCEL (Microsoft, US). Values of $p < 0.05$ were taken to be significant in the four experiments.

RESULTS

Experiment I - Shifting from a deficient to a balanced diet and vice-versa - The influence of the type of diet on host nutritional status was quite evident, mice ingesting the balanced diet permanently (group CG4) or temporarily (groups G1, G2) showing the best performance regarding weight gain (Fig. 1). Food intake curves showed good correlation with body weight gain.

Studies on gross pathology detected a higher mean liver weight for the "early undernourished" mice (group I), but differences regarding spleen weights between groups G1 and G2 were not significant.

The histologic picture presented by mice in groups CG3 and G1 did not substantially differ. It included scattered small periportal granulomas, with variable amount of peri-sinusoidal and septal fibrosis and mild signs of non-specific reactionary hepatitis (mononuclear leukocytes admixed with few polymorphonuclear eosinophils in portal spaces and around central veins). A few isolated foci of ischemic necrosis or moderate fatty changes were sometimes observed. No instance of portal concentration of granulomas, associated with portal expansion and fibrotic connections between portal spaces (“pipestem”-like fibrosis) was recorded. Well-nourished animals (group CG-4) also presented scattered, small periportal granulomas

of variable sizes and cellular composition. In some of them, there was a large amount of lymphocytes and eosinophils, while in others fibroblasts and macrophages predominated. Granuloma clusters in portal spaces were frequently observed, but the clear-cut picture of “pipestem”-like fibrosis did not develop. Besides focal cellular infiltrations in portal, parenchymal, and centrilobular areas, there were also foci of coagulative necrosis and periportal or diffuse fatty changes. After shifting to a deficient diet (group G-2), the histologic picture underwent mild to moderate changes: several granulomas appeared large, round, with fusiform cells within a loose fibrillar matrix (Fig. 3), subsiding the signs of non-reactive hepatitis. The

Fig. 1

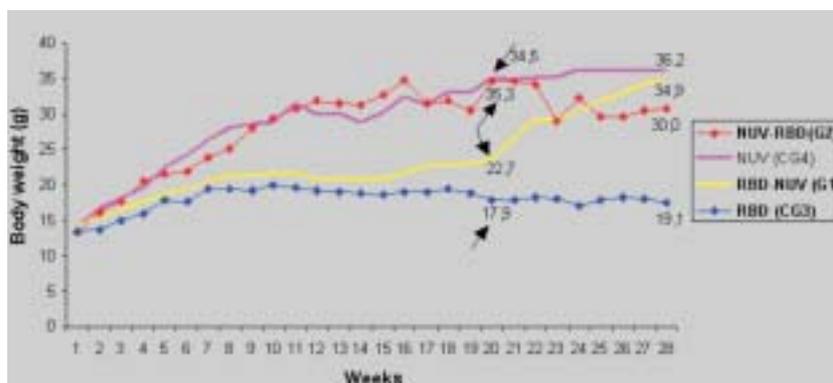


Fig. 2

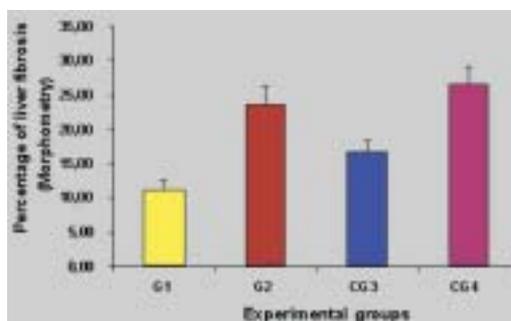


Fig. 3

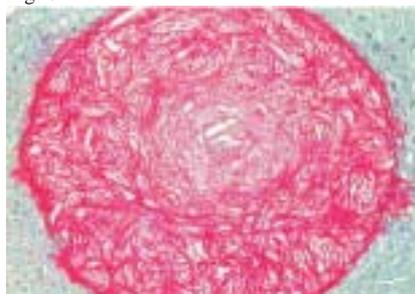
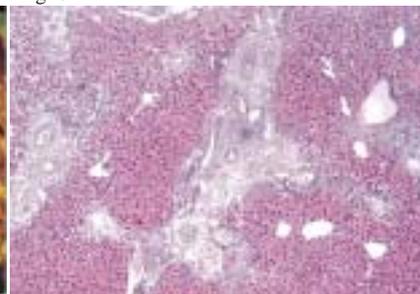


Fig. 4



Fig. 5



Experiment I - Fig. 1: body weight of mice infected with *Schistosoma mansoni* submitted to “early” (G1) and/or “late” undernutrition (G2), as compared to their respective controls (CG3 and CG4). Arrows indicate the mean body weight of the mice at the time point of diet shifting, in groups G1 and G2; and the evolution of the growth curves from this time point onward, in “long-lasting” undernourished (CG3) and “long-lasting” normally fed (CG4) control groups. Fig. 2: morphometric evaluation on the amount of fibrous tissue (collagen) in the liver of *S. mansoni* infected mice submitted to either “early” and/or “late” undernutrition in comparison to controls. G1: early undernutrition; G2: late undernutrition; CG3: “long-lasting” undernutrition; CG4: “long-lasting” normal feeding. Fig. 3: enlarged fibrotic granuloma found in “late undernourished” mice, illustrating its size, cellular distribution and collagen whorled disposition. Picrosirius red x 200. Fig. 4: cut section of human liver with periportal schistosomal fibrosis (“clay pipestem” or Symmers’ fibrosis), showing increase of fibrous tissue and amplification of portal spaces on a background of normal-looking parenchyma. Fig. 5: schistosomal periportal liver fibrosis in the murine model, showing periportal granulomas, fibrous enlargement, increased vascularization, and inflammatory infiltration of the portal space. Hematoxylin and Eosin x 50

“pipestem”-like fibrosis (Figs 4, 5) was detected in two mice belonging to the “late undernourished” group (G2).

Morphometric analysis showed that in the well-nourished controls (Group CG4) and “late undernourished” mice (group G2), the volume density and the mean size of periportal granulomas were significantly higher in comparison with the other groups.

Total liver fibrosis measured morphometrically as percentage of hepatic tissue (Fig. 2) and biochemically as hydroxyproline in liver tissue homogenates showed good correlation by both methods of evaluation, malnourished groups having the worst performance.

Experiment II - Repeated infections - The liver and spleen/body weight ratios from undernourished mice were always lower than those from their counterparts (reinfected well-nourished mice), as seen in Fig. 6.

Histologic examination detected in undernourished single infected mice (A1 group) only scattered small periportal granulomas and mild non-specific reactional hepatitis (mononuclear and polymorphonuclear eosinophils in some portal spaces and around central veins). Among well-nourished single infected mice only one animal developed the “pipestem” lesion, but in reinfected mice images of portal concentration of circumoval granulomas causing fibrotic expansion and development of thin fibrous tracts connecting portal spaces (murine “pipestem”-like fibrosis) were seen in four mice (Fig. 7) and in three additional animals a mixed histologic picture was detected (scattered circumoval granulomas and “pipestem”-like fibrosis). Reinfected undernourished mice, however, were not able to develop “pipestem”-like fibrosis, although thin fibrous strands could eventually be seen from one portal space to the other.

Morphometric and biochemical evaluations regarding the amount of collagen deposited in the liver showed excellent correlation between the two methods, higher concentrations of fibrous tissue having been detected in the reinfected groups. (Fig. 9A, B).

Experiment III - Genetic background (inbred mice from BALB/c and C57BL/10 strains) - In all mice strains (inbred and outbred), the liver and spleen/body weight ratios were higher in the well-nourished groups.

In undernourished BALB/c mice, scattered small fibrotic granulomas around disintegrating miracidia or only fragments of *S. mansoni* eggshells were seen on histologic examination. Some polymorphonuclear cells and macrophages could sometimes be found at the periphery of the granulomatous reaction. Thin fibrous tracts were eventually found connecting granulomas to each other or linking them to portal spaces. No reactional hepatitis was present, but small foci of ischemic necrosis and some degree of periportal fatty metamorphosis were detected in a few instances. (Fig. 10).

In undernourished mice of the C 57BL/10 strain the granulomas seemed to be more cellular and collagen deposition less abundant than in the BALB/c mouse strain. Reactive hepatitis was intense, the acute inflammatory cells invading not only the portal spaces but also the sinusoids (polymorphonuclear cells including eosinophils). Collagen deposition had a fibrillar aspect and low density. “Pipestem”-like murine fibrosis was detected in two animals (Fig. 11) and in two other mice a mixed lesion

(scattered granulomas and fibrous enlargement of portal spaces with fibrous connections with some granulomas) suggested the onset of “pipestem”-like lesions.

The livers from well-nourished BALB/c inbred mice and Swiss outbred controls did not significantly differ in their general histologic appearance. Foci of ischemic necrosis, acute reactive hepatitis of variable intensity and collagenized circumoval granulomas were the most outstanding features of the histopathologic picture. In well-nourished C57BL/10 mice, fibrous tracts between granulomas and portal spaces and reactive hepatitis seemed to occur in a higher frequency.

Morphometric evaluation (Fig. 12A, B, C, D) detected that undernourished inbred C57BL/10 mice had higher concentrations of total liver collagen than undernourished outbred Swiss mice. Significant results were also found between well-nourished inbred mice of both strains and undernourished Swiss outbred controls. Morphometric studies showed that granuloma size and volume density were greater in well-nourished mice, but granuloma numerical density was higher in undernourished animals in both inbred and outbred mouse strains.

Experiment IV - Immunological response - Preliminary results based upon immunological studies in outbred Swiss mice of both sexes, showed that the lymphoproliferative response (spleen cultured cells stimulated with Con-A and SEA) and the production of the cytokines IFN- γ and IL-5 in undernourished infected mice did not differ from those of well-nourished controls. However, the serum levels of SEA-specific antibodies IgG1, IgG2a, IgG2b, and IgG3 in undernourished mice were two to four-fold lower than those detected for well-nourished control animals (Fig. 13).

DISCUSSION

Although it is assumed that the chimpanzee and the baboon (Farah et al. 2000) models most closely mimic the pathogenesis of Symmers' fibrosis observed in man than does the mouse model, the use of the latest is relevant and widespread for several reasons, offering a convenient tool for examination of genetic regulation of schistosomal hepatic fibrosis and for elucidation of mechanisms of hepatic fibrosis in human and experimental hosts.

Undernourished mice have proved to be unable to develop the morphologic picture of “pipestem” periportal-like fibrosis (Coutinho et al. 1997) described in several investigations on the murine model (DeWitt & Warren 1959, Andrade & Warren 1964, Cheever 1965, Warren 1966, Andrade & Cheever 1993, Henderson et al 1993, Andrade et al. 1997). In this paper experimental attempts designed to explain the non-occurrence of the lesion in undernourished mice are reported.

Shifting from a deficient to a balanced diet was not able to induce the development of the lesion in outbred Swiss mice infected with *S. mansoni*. However, in animals submitted to “late undernutrition”, two mice developed “pipestem”-like fibrosis and it was noticed the presence of conspicuous large fibrotic granulomas, indicating that they were probably formed when the animals were still well-nourished, but collagen failed to be degraded later on, when mice ingested a deficient diet.

The way undernutrition can interfere with the process of fibrous resorption deserves further investigation. It is known that the rate of collagen degradation depends on several known and unknown factors, among them the interplay of collagenase and tissue collagenase inhibitors (Truden & Boros 1988) and the degree of collagen maturation or “cross-linkings” (Ricard-Blum et al. 1992). In this way, nutrition may interfere in all these steps, once protein synthesis occur in all these processes. For this reason, the host nutritional status may be considered as a contributory factor to the remodeling of periportal granulomas of hepatic schistosomiasis and schistosomal granuloma in undernourished mice can be taken as an adequate model for studies on the dynamics

of hepatic fibrosis (Coutinho et al. 2003).

Collagen deposition seemed to be increased in well-nourished mice and significantly reduced in undernourished animals where it appeared looser and more fibrillar than in controls. Repeated infections lead to a higher collagen concentration in the liver of undernourished and well nourished mice, higher values being ascribed to well-nourished controls. These results were detected by both morphometric and biochemical evaluations and confirmed by histological examination. In spite of this, no case of “pipestem”-like periportal fibrosis was seen in undernourished outbred Swiss mice, even after repeated infections. However, seven out of 16 well-nourished Swiss mice developed the lesion.

Fig. 6

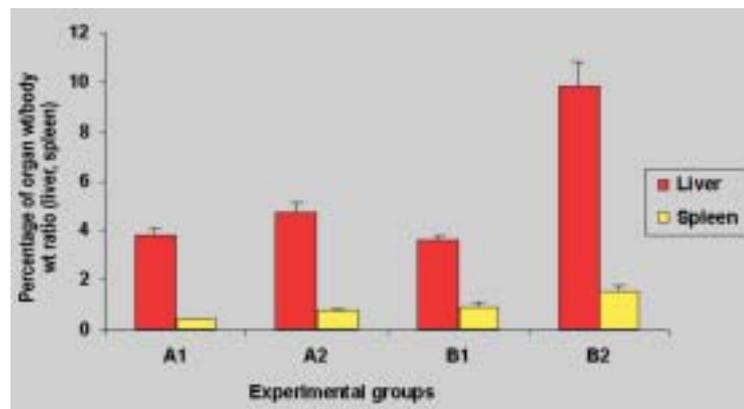


Fig. 7

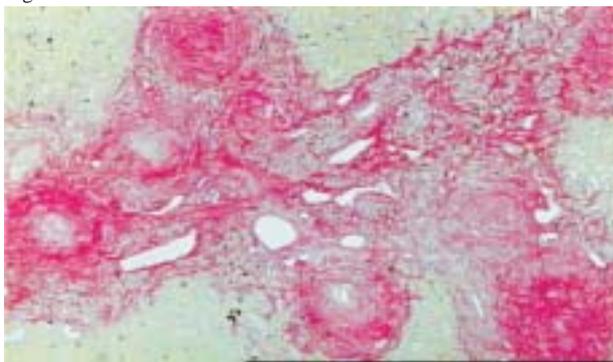
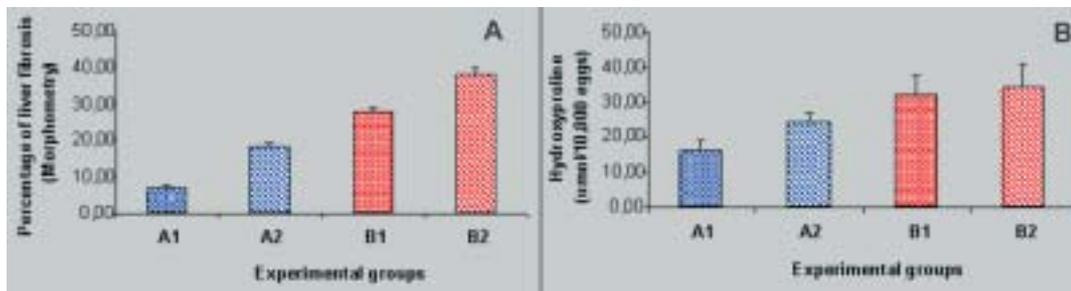


Fig. 8



Fig. 9



Experiment II - Fig. 6: organomegaly (liver, spleen) in undernourished and well-nourished mice submitted to single and/or repeated infections with *Schistosoma mansoni*. A1: undernourished (single infection); A2: well nourished (single infection); B1: undernourished (reinfected); B2: well nourished (reinfected). Fig. 7: “pipestem”-like liver fibrosis with vascular neoformation, in well-nourished reinfected Swiss mouse. Picrosirius x 100. Fig. 8: scattered small periportal granulomas connected by thin fibrous bridges, in undernourished reinfected Swiss mouse. Picrosirius red x 50. Fig. 9: morphometric and biochemical evaluations on the amount of hydroxyproline (collagen) in the liver of undernourished and well-nourished mice submitted to single and/or repeated infections with *S. mansoni*. A1: undernourished (single infection); A2: well-nourished (single infection); B1: undernourished (reinfected); B2: well-nourished (reinfected)

Liver and spleen/body weight ratios, in the four reported trials, showed higher values for the well-nourished groups, being closely related to the type of diet ingested by the animal (i.e., deficient or balanced diet). By morphometric evaluation, considered to be a most sensitive method (Barbosa Jr. 2001), undernourished inbred mice of the C57BL/10 strain had higher concentrations of total liver collagen than undernourished Swiss (outbred) con-

trols. In those mice, the signs of reactive hepatitis were more intense, numerous granulomas were still in the exsudative acute phase, the deposited collagen was loose and fibrillar in appearance. The unexpected finding of “pipestem”-like fibrosis occurring in four out of 21 undernourished animals still rests unexplained. The influence of another parasite strain (SLM strain-PE) different from the one used in the other three experiments (BH strain-

Fig. 10

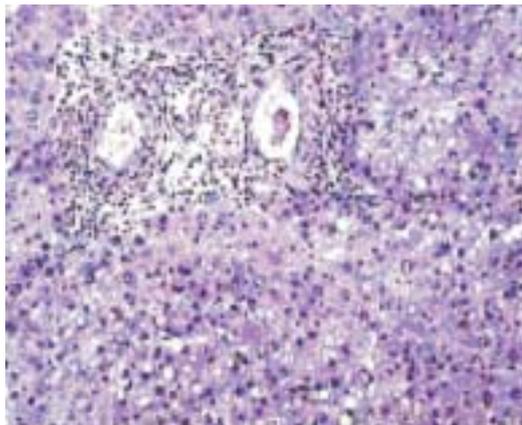


Fig. 11

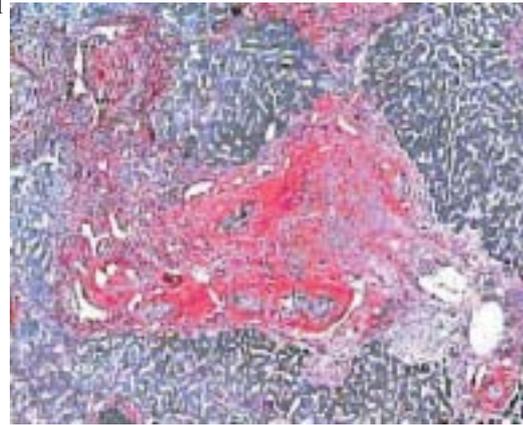
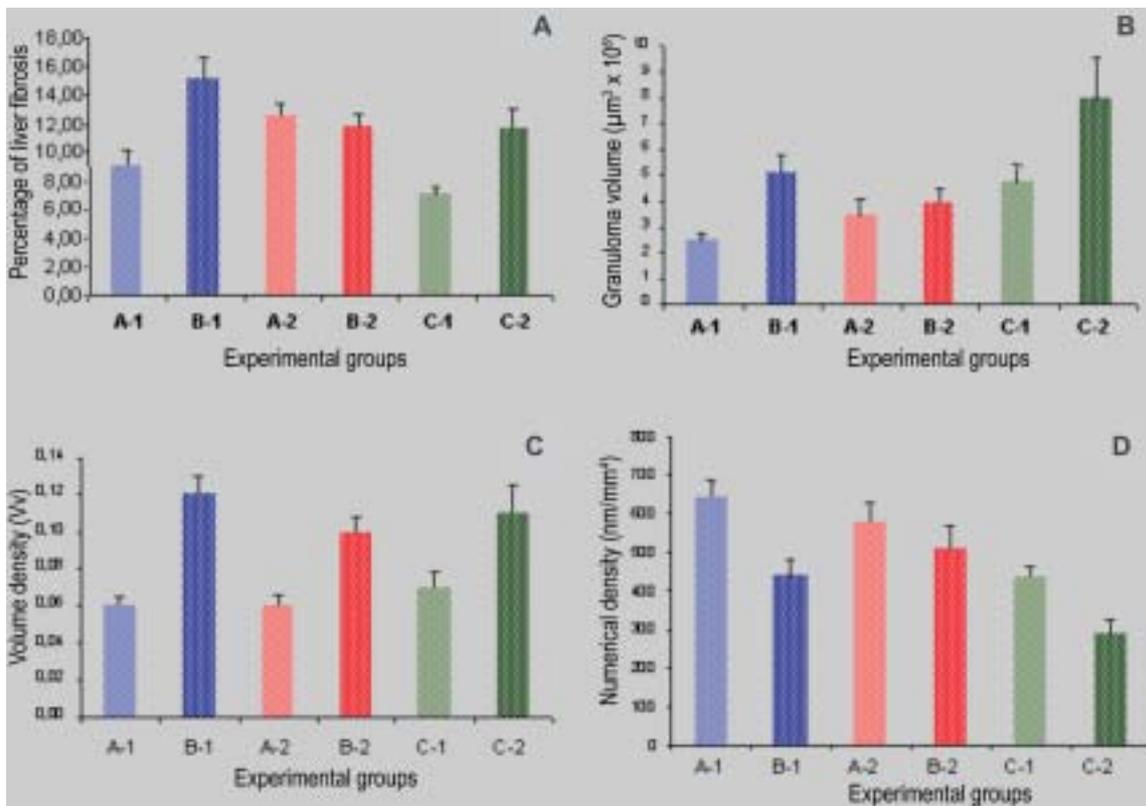
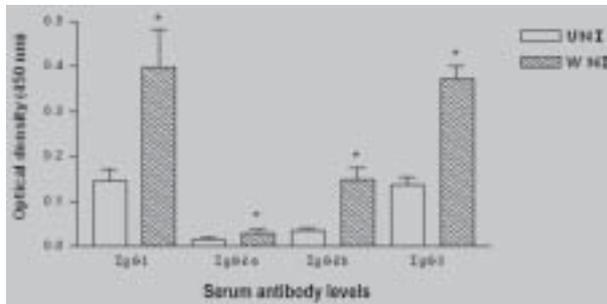


Fig. 12



Experiment III - Fig. 10: scattered small sized cellular granulomas and periportal fatty changes in the liver of undernourished BALB/c mouse. H & E x 100. Fig. 11: “pipestem”-like lesion in the liver of a C57BL/10 mouse, showing fibrous enlargement, telangiectasia and periportal granulomas in a portal space. Picosirius red x 100. Fig. 12: morphometric parameters (percentage of hepatic fibrous tissue, granuloma size, volume, and numerical densities of circumoval granulomas) in the liver of undernourished and well-nourished BALB/c and C57BL/10 inbred mice infected with *Schistosoma mansoni*, in comparison with outbred controls. A1: undernourished BALB/c mice; B1: well-nourished C57BL/10 mice; A2: undernourished BALB/c mice; B2: well-nourished C57BL/10 mice; C1: undernourished Swiss mice (outbred); C2: well-nourished Swiss mice (outbred)



Experiment IV - Fig. 13: titers of SEA-specific antibodies in *Schistosoma mansoni* infected mice, obtained four months after a single infection. UNI: undernourished infected mice; WNI: well-nourished infected mice. Data represent the mean \pm SD of 7-8 mice per group.

MG), due to a laboratory accident occurring during the trial, cannot be discarded. Studies are now being carried out focusing on the role of different parasite strains in the pathogenesis of the lesion in undernourished mice.

In Experiment III, inbred BALB/c mice had the worst performance regarding growth curves and none of the mice, in either diet, developed the murine "pipestem"-like periportal fibrosis. This is in disagreement with Flannery's observations (2003), who mentions that 59% of well nourished BALB/c mice developed "pipestem" fibrosis upon *S. mansoni* infection. However, in the reported studies the duration of infection was longer (20-24 weeks) and this may explain the lack of success in reproducing the lesion in our own trial.

The influence of genetic factors on the manifestations of disease associated with infection with *S. mansoni* (portal hypertension, liver granulomas, hepato-splenomegaly) and their modulation were studied in inbred strains of mice by Fanning et al. (1981). Granuloma size, organomegaly, and portal venous pressure have proven to be strain dependent and determined by more than one gene. According to these authors, disease associated with infection with *S. mansoni* and its modulation in mice are influenced by the genetic (non- H-2) background of the host and dependent in part on cell-mediated immunity.

Body, liver, and spleen weights seem to vary among different strains. C3H/HeJ mice were found to develop the most pronounced degree of hepatosplenomegaly and granuloma formation. (Fanning et al. 1981). According to these investigators, more than one gene appears to be involved in the inheritance of the propensity to develop severe disease.

Hepatic fibrosis in *S. mansoni* infected mice is mainly associated with the circumoval granulomas (Dunn et al 1977), although some portal fibrosis not associated with granulomas is also present (Cheever 1965).

Marked differences in granuloma volume and in hepatic fibrosis were found between strains of well-nourished mice (Dunn 1980, Cheever et al. 1987). Besides, those strains with the largest granulomas also showed the most hepatic fibrosis. According to the last author, the regulation of granuloma size and of hepatic fibrosis is clearly complex and involves genes both outside of and within

the major histocompatibility complex. Studies on differences in hepatic fibrosis in outbred ICR mice and several inbred mice strains by Cheever et al. (1983) showed that hepatic fibrosis was much more marked in *S. mansoni* infected mice of an outbred ICR strain.

In this investigation, decreased total liver fibrosis observed in undernourished infected mice may have resulted from an impaired mechanism of repair, a lower antigen load or a low antibody production due to low protein synthesis in a low-protein fed host (Oliveira et al. 2004).

Although the lymphoproliferative response and the cytokine production (IFN- γ and IL-5) from undernourished outbred infected mice had been similar to those of well-nourished controls, the humoral immune response based on the serum levels of SEA-specific antibodies obtained in Experiment IV, coupled to the gathered morphological, morphometric, and biochemical data, suggest that the host nutritional status may interfere in the connective tissue changes occurring in hepatic schistosomiasis.

ACKNOWLEDGMENTS

To Zilton A Andrade, Aryon A Barbosa Jr., Ricardo Ribeiro dos Santos, Milena BP Soares, Sheilla A Oliveira, Luciana S Flannery, Andréia F Barros, Fabiana I Silva, Sandra L Carvalho, Claudia L Cavalcanti, and Carlos F Luna for their valuable participation in different steps of the experiments here reported, and to Mr Roni Evencio de Araújo, for skilled technical assistance.

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