DETERMINATION OF MACROPHAGE ACTIVITY IN ALBINO MICE EXPERIMENTALLY INFECTED WITH *CYSTOISOSPORA FELIS* (WENYON, 1923) FRENKEL, 1977 (APICOMPLEXA: SARCOCYSTIDAE)

RONALD BASTOS FREIRE[1] & CARLOS WILSON LOPES[2]

(1) Departamento de Microbiologia e Imunologia Veterinária (2) Departamento de Parasitologia Animal, IB. Universidade Federal Rural do Rio de Janeiro. Km 47 da antiga estrada Rio-São Paulo. 23.851-970 Seropédica - RJ Brasil. Projeto Sanidade Animal (EMBRAPA/UFRRJ).

SUMMARY: Blood stream clearance of nitro blue tetrazolium has been evaluated to establish the phagocytic activity of macrophages from albino mice experimentally infected with 10^6 oocysts of *Cystoisospora felis*. Spleen and liver per cent uptakes were calculated, according to the relationship of the combined weight of liver, and spleen, and the fraction of test material removed from circulation per minute. A temporary blockade of peritoneal macrophages, before infection with 103 sporozoites by the- same inoculation route, was also experienced in order to determine the phagocytosis influence on spreading of *C. felis* hipnozoites, in the host's viscera. Although no variations occurred in the per cent uptake of particles, by the liver and spleen of infected animals, macrophage activation takes place, following the oocyst infection. The macrophage supression in mice inoculated intraperitoneally with 1% activated charcoal, was sufficient to prevent sporozoites infection and, hipnozoites detection in peptic digests of mesenteric lymph nodes, liver and spleen.

KEY WORDS: *Cystoisospora felis,* macrophages, infection.

INTRODUCTION

Functionally, macrophages are a diverse group of cells, acting at many levels in their response to foreign materials. They are important in the development and regulation of the immune response since they can act both as accessory cells and suppresser cells. In addition, macrophages appear to play a role in the resistance of the host to the attack by parasites and a variety of microorganisms. The removal of particulate materials from the circulation is the function of the phagocyte cells lining the blood sinuses, mainly those in the liver, spleen and bone marrow. Among these organs the liver is, according to its size, the main site of particle localization (HERSCOWITZ *et alii,* 1981). Because of the high uptake by the liver, measurements of blood clearance rates generally reflect the activity of the macrophage population of this organ. Localization in the spleen, as well as bone marrow and lungs, can also be influenced by the activity of liver macrophages (LIMA *et alii,* 1977). Since *Cystoisospora felis* presents a tropism to mesenteric lymph nodes, spleen and liver (FREIRE, 1993), its influence on the blood stream clearance of particles has been used as a parameter of phagocytic activity of macrophages from experimentally infected albino mice. Suppression of phagocytosis *in vitro*, after injection of activated charcoal particles, causing temporary blockade, has also been induced to know about the importance of phagocytosis on spreading of hipnozoites following sporozoite injection in the peritoneal cavity of albino mice.

MATERIALS AND METHODS

Animals

One hundred male Swiss western mice, weighing 15-20 g from the colony of the Immunology Section of the Centro de Sanidade Animal EMBRAPA/UFRRJ were housed in controlled environment and provided with fresh-water and commercial mouse diet *ad libitum.*

Parasites

Oocysts, sporozoites and hipnozoites of C. *felis* were obtained, quantified and evaluated for viability by methodologies previously described (FREIRE, 1993).

Phagocytic activity

Blood stream removal of particulate material was achieved by the determination of the rate of blood clearance of nitro blue tetrazolium (NBT) in 35 albino mice, experimentally infected with 10^6 sporulated oocysts of *C. felis* and 35 non infected control mice. Following infection, groups of five animals were anesthetized and bled at 3, 6, 12, 15, 30 and 45 days post infection (DPI). The procedure has been repeated every 5 - 10 minutes, until obtaining five blood samples per each day of experiment. Aliquots of 0.75 ml of citrated blood into test tubes containing 5 l of 1 mg.ml^{-1} bacterial endotoxin (IBI, Glessen, Germany) were incubated at 37°C during 10 minutes, and added 60 l of 0.4 mM NBT and 340 nM sucrose. The contents of each tube was sifted across a rayon-wool column (Santista, SP., Brazil) which was washed twice with 0.15 M phosphate buffered saline (PBS) pH 7.2 (NaCl 8 gl^{-1}, KCl 0.2 gl^{-1}, Na2HP0$_4$ 1.15 gl^{-1}- KH2PO44 0.2 gl^{-1}). The adherent macrophages were trapped by the rayon-wool and added of 1 ml of dioxane (Merck, USA) and incubated at 10° C during 20 minutes. At the end of the experiment the absorbance of the samples was measured at 460 nm in a spectrophotometer (Bausch & Lomb, Germany). The values of absorbance for individual animals were plotted against time of sampling and the rate of clearance among infected and control mice compared. The per cent uptake of NBT in the liver was estimated according to the quantity of the test material removed from circulation per minute and the ratio of liver and spleen weights as described elsewhere (HERSCOWITZ *et alii*, 1981).

Macrophage blockade and sporozoite infection

Two groups of 20 albino mice were infected with 10^3 sporozoites of *C. felis* by intraperitoneal inoculation. The first 20 animals were previously injected with activated charcoal particles (0.1% in PBS), two hours before infection with sporozoites using the same route of inoculation. Five animals from each group were killed at 1, 3, 5, and 7 DPI and the hipnozoites that were present in mesenteric lymph nodes, spleen and liver, were quantified.

RESULTS AND DISCUSSION

One of the earliest functions of macrophages is their ability to take up particulate materials by phagocytosis. It has also been known that macrophages from animals infected with bacteria and parasites are morphologically and functionally modified. These alterations include tumoricidal, increased microbicidal and phagocytic activities (HERSCOWITZ *et alii*, 1981; FAYER *et alii*, 1988; FREIRE *et alii*,1991). Parasitic agents such as *Toxoplasma* and *Besnoitia* can induce the activated macrophages (HERSCOWITZ *et alii*, 1981). Activated macrophages perform certain functions more efficiently than their normal resident counterparts. These functions may include intracellular microbicidal capacity, and *in vitro* phagocytic capability.

Since *C. felis* hipnozoites infect mesenteric lymph nodes, spleen and liver (FREIRE, 1993), their influence on phagocytic removal of particles from the circulation was evaluated. Albino mice were inoculated with an oral dose of 10^6 sporulated oocysts of *C. felis*. Although most hipnozoites have been recovered at 3, 6, 12, 15, 20, 30 and 45 days post infection (DPI) from the viscera of infected animals, immunological negative modulation has not been shown when NBT uptake was measured in the blood stream of infected mice, comparatively to non infected controls (Table 1).

Table 1- Macrophage activity[1] in albino mice experimentally inoculated with 10^6 oocysts of *Cystoisospora felis*

Days post infection	Percentage of clearance[2]
3	98.3
6	103.0
12	99.1
15	100.0
20	105.8
30	100.0
45	88.7
Control[3]	100.0

[1] NBT exclusion test.
[2] Results are a mean of five observations.
[3] Results are a mean of five control animals for each day of experiment.

Since the blood clearance of particles can be used as an indicative of spleen and liver activities (HERSCOWITZ *et alii*, 1981), the per cent uptake of NBT for these organs was estimated (Table 2). Results showed a proportional activity to the spleen and liver phagocytes, with NBT uptake varying from 2.3% to 7.1% in the spleen and 71.9% to 90.1% in the liver. Although liver macrophage activity decreased at 12 and 30 DPI it was always superior to the results achieved for the control animals, suggesting that occurred a macrophage activation after *C. felis* oocysts infection.

Table 2 - Per cent uptake of NBT in the liver and spleen of albino mice infected with 10^6 oocysts of *Cystoisospora felis*

Days post infection	Percentage of injected dose[1]	
	Liver	Spleen
3	89.7	2.8
6	90.1	2.3
12	71.9	5.9
15	88.6	2.3
20	88.5	2.4
30	72.3	7.1
45	80.5	2.8
Control[2]	71.3	4.4

[1] Results are a mean of five observations.
[2] Results are a mean of 35 observations.

The blockade of macrophage activities with activated charcoal was sufficient to avoid infection of mice by 10^3 sporozoites of *C. felis* using the same route (Table 3). The control group of animals that did not receive the carbon treatment produced most hipnozoites, quantified in mesenteric lymph nodes, spleen and liver. The sporozoites infection inhibition corroborates with previous experiments done with *Isospora lacazei* (HERNANDEZ *et alii*, 1978) were it was demonstrated the necessity of the phagocytosis for the parasite spreading across the host's organism. Similarly, phagocytosis have been demonstrated to be fundamental for C. *felis* extraintestinal life cycle development.

Table 3 - Hipnozoites recovered by peptic digestion of organs[1] from albino mice infected with 10^3 sporozoites of *Cystoisospora felis*

Animal group	Hipnozoites recovered[2] Days post infection			
	13	5	7	
A[3]	0	0	0	0
B[4]	$0.7 \times 10^3 \pm 0.10$	$2.2 \times 10^3 \pm 0.22$	$3.4 \times 10^3 \pm 0.50$	$1.04 \times 10^4 \pm 0.11$

[1] Peptic digestion of mesenteric lymph nodes, spleen and liver.
[2] Results are a mean of five observations.
[3] Animals infected previously treated with 0,1% activated charcoal.
[4] Infected animals, without treatment with activated charcoal.

SUMÁRIO

110 camundongos suíço-albinos, pesando entre 12 e 15g, foram submetidos a ensaios visando estabelecer a atividade fagocítica de macrófagos circulantes, assim como sua influência na disseminação de formas extra-intestinais de *Cystoisospora felis* no organismo de hospedeiros intermediários. A atividade fagocítica de fagócitos foi determinada através do índice de clarificação de partículas de azul de tetrazólio (NBT) no sangue circulante de camundongos experimentalmente inoculados com 10^6 oocistos de C. *felis*, por via oral. O percentual de captação hepática e esplênica foi calculado em relação ao peso relativo do fígado e do baço de cada animal e a quantidade de partículas de NBT clarificadas por minuto. O bloqueio da atividade fagocitária de fagócitos peritoneais pela inoculação de partículas de carvão vegetal duas horas antes da infecção de esporozoitas, por via intraperitoneal, foi capaz de impedir a infecção e conseqüente formação de hipnozoitas, os quais não foram detectados nos órgãos de predileção do parasita como linfonodos mesentéricos, baço ou fígado. Controles não-suprimidos apresentaram quantidades relativamente grandes de formas císticas extra-intestinais, após digestão péptica de suas vísceras. Embora nenhuma alteração tenha se manifestado na captação de partículas, ao nível de fígado e baço dos animais infectados, ocorreu ativação nos fagócitos circulantes, após inoculação de oocistos de *C. felis*.

PALAVRAS-CHAVE: *Cystoisospora felis*, macrófagos, infecção.

REFERENCES

FAYER, R., ANDREWS, C. & DUBEY, J.P. (1988). Lysate of *Sarcocystis cruzi* bradizoites stimulates RAW 26.7 macrophages to produce tumor necrosis factor (Cachectin). *J. Parasitol., 74*:660-664.

FREIRE, R.B. (1993). Cystoisospora felis *(Wenyon, 1926) Frenkel, 1977 (Apicomplexa: Sarcocystidae): caracterização morfológica, infecção experimental e resposta imune em camundongos albinos.* Tese de Doutorado, UFRRJ, 110 p.

FREIRE, R.B., PIRES, P.P., DE SÁ, W.F. & LOPES, C.W.G. (1991). Detecção de fator de supressão no soro de bovinos experimentalmente infectados com *Sarcocystis cruzi* (Hasselman, 1923) Wenyon, 1926 (Apicomplexa: Sarcocystidae). *Arq. Univ. Fed. Rural do Rio de Janeiro, 13*:119-130.

HERNANDEZ, S., NAVARRETE, R., CALERO, R., BELERRA, C. & MARTINEZ, F. (1978). Electron microscopical study of extraintestinal stagies of *Isospora lacazei* in *Carduelis carduelis*. 4th Intern. Congr. Parasitol., Warsaw, *8*:19-26.

HERSCOWITZ, H.B., HOLDEN, H.T., BELLANTI, J.A. & GHAFFAR, A. (1981). *Manual of macrophage methodology: collection, characterization and function.* Noel Rose Ed., New York, USA, 531 p.

LIMA, A.P., SOARES, J.B., GRECO, J.B., GALIZZI, J. & LANÇADO, J.R. (1977). *Métodos de Laboratório Aplicados à Clínica.* Guanabara Koogan Ed. Brasil, 669 p.

(Received 27 December 1993, Accepted 17 August 1995)