Acta Veterinaria (Beograd), Vol. 49. No. 1, 21–28, 1999.

UDK 619:616.993.1

CHANGES IN WHITE BLOOD CELL COUNT AND FORMATION OF LESIONS IN MICE EXPERIMENTALLY INFECTED WITH ENCEPHALITOZOON CUNICULI

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(Received, 8. December 1998.)

White blood cell count (WBC) and formation of histological lesions were evaluated in mice experimentally infected with E. cuniculi during 120 days of observation.

WBC showed a significant decrease of some leukocytes including lymphocytes at 10, 30, 60, and 90 days after infection. On the other hand the number of monocytes was increased in the second half of the experiment. Lesion developed earliest in the liver. Later on other organs studied (brain, lung, kidney and heart) were showen to be infected in the acute stage of infection. Some relationship between changes in WBC and movement of these cells into infected tissues was inferred.

Key words: White blood cells, histological lesions, mice, E. cuniculi

INTRODUCTION

Encephalitozoonosis is caused by the intracellular protozoan *Encephalitozoon cuniculi.* The parasite can infect many animal species and has been reported in humans, as well (Sprague et al. 1992).

Immunologically competent hosts that are naturally or experimentally infected with *E. cuniculi* usually express few clinical signs of disease. Persistence of the parasite produces only chronic asymptomatic infections. Many studies on the pathology of encephalitozoonosis have revealed that the central nervous system and kidney are common target organs for this infection, although many

different cells, tissues, and organs can be infected by the parasite (Levkut et al., 1997).

On the other hand, immunodeficient hosts, such as severe combined immunodeficient mice (SCID), develop lethal disease after experimental infection (Koudela et al., 1993). These animals are deficient in T-and B-lymphocytes that consitute the specific immune system, white the innate immune system, consisting primarily of macrophages, natural killer cells, and neutrophils is not altered (Seydel and Stanley, 1996).

The purpose of this study was to examine early changes of haematological values in association with the development of tissue lesions in mice experimentally infected with *E. cuniculi.*

MATERIALS AND METHODS

Animals. Seventy-two 8 week-old mice from an inbred conventional mouse colony (C57 BL6) were used. The mice were housed at 22 $\pm$ 2^{0}C under natural-light conditions, humidity 45% and had free access to tap water and commercially available stock food (Feedstuff P, Top Dovo, Dobra Voda).The mice were divided into two groups of thirty-six each and kept in plastic cages with 18 males and 18 females in each group separately. They were negative for encephalitozoonosis.

Sera. Serum was collected after cardiac puncture for 0.5-1 ml of blood from each animal. The sera obtained from the blood samples were frozen and maintained at -20^{0}C until used.

Organisms. A murine isolate of *Encephalitozoon cuniculi* was grown in "E6" vero cells (green monkey kidney cells). The cells were cultivated in modified RPMI 1640 medium supplemented with 5% foetal calf serum. Spores freshly collected from the culture supernatans according to Hipikova et al. (1995) were used for aniimal inoculation.

Inoculation. Thirty-six mice of both sexes were inoculated intraperitoneally with 2.5 x 10^7 spores of *E. cuniculi* in a single dose (0.5 ml) in phosphate-buffered saline (PBS). Thirty-six mice were used as the control. Six mice from each group were killed at intervals (10, 20, 30, 60,90, 120 days during the experiment.

White blood cell count. Blood was obtained by cardiac puncture following ether anasthesia and transferred into Turk's solution. The cells were then counted in a haemocytometer. Differential cell counts were made on blood smears after May-Grünwald-Giemsa staining by counting 100 cells per slide.

Computation for absolute leukocyte counts. Absolute leukocyte counts (Table 2) were computed as follows: WBC count x% of different types of leukocytes.

Serological examination. Sera were tested by indirect immunofluorescence for the presence of antibodies to *E. cuniculi.* The animals whose sera reacted at a dilution of 1:64 or higher were considered to be positive. The titres were expressed as the highest serum dilution giving bright staining of the spore body as described previously (Chalupsky et al., 1973). The conjugate used was a fluorescein-conjugated swine anti-mouse globulin (SEVAC, Prague, Szech Republic).

Necropsy. Samples of liver, lung, kidney, brain and heart were taken for histopathological examination at 10, 30, 60, 90 and 120 days after infection. The preparation and microscopical evaluation were carried out on six histological sections taken from two localities in each organ and processed in a standard manner, i. e. fixed in 10% neutral formalin and embedded in paraffin. Histological sections of 5-6 μm thickness were stained with haematoxylin-eosin and Giemsa stain.

RESULTS

Animals infected with E. cuniculi were seropositive at 1:64 on day 10 after infection (Table 1). The highest titre of antibodies was observed at day 60. The last examination of serum in the experiment again showed antibody titres 1:64.

Table 1. Antibody response and lesions in *E. cuniculi* infected animals

days after infection	titration of antibodies	microscopic lesions				
		liver	lung	brain	kidney	hearth
10 days	1:64	+	–	–	–	–
30 days	1:64	+	+	+	–	–
60 days	1:256	+	+	+	–	–
90 days	1:128	+	+	+	+	–
120 days	1:64	+	+	+	+	+

+ lesions observed; - no lesions

WBC count showed a significant decrease of leukocytes (Table1) at days 10, 20, 30, 60 and 90 of the experiment in the infected groups. Lymphocytes included in the group of leukocytes were similarly significantly decreased in the experimental gruop at day 10, 20, 30, 60 and 90 of the experiment. Neutrophils showed significant decreases at days 60 and 90 of the experiment in the infected group. A significant increase of monocytes in the infected group was seen in the second half of the experiment (60, 90 and 120 d. p. i.) The number of eosinophils was not significantly changed during the experiment in either group.

The findings at necropsy (Table 1) consisted of focal lymphocytic infiltration or formation of microgranulomatous nodules. Lesions were observed first in the liver (Figure 1) later in the brain (Figure 2) and lung. The two last examinations showed the occurrence of lesions also in the kidney and the last examination included lesions in the heart as well.

Table 2. Haematological values in control mice and mice infected with E. cuniculi

Days after infection		Leukocytes	Lymphocytes	Neutrophils	Monocytes	Eosinophils
10	e	4.00 ± 0.64	3.04 ± 0.68 (75.33%)	0.75 ± 0.31 (19.67%)	0.16 ± 0.09 (3.67%)	0.08 ± 0.01 (0.67%)
	c	10.50 ± 4.50[a]	8.20 ± 350[a] (79.00%)	1.03 ± 0.62 (10.00%)	0.12 ± 0.17 (1.50%)	0.00 ± 0.00 (0.00%)
20	e	3.46 ± 1.36	2.86 ± 1.26 (81.20%)	0.46 ± 0.25 (13.80%)	0.15 ± 0.06 (5.00%)	0.00 ± 0.00 (0.00%)
	c	10.20 ± 4.30[a]	8.00 ± 3.40[a] (79.00%)	0.75 ± 0.37 (9.60%)	0.15 ± 0.18 (1.80%)	0.00 ± 0.00 (0.00%)
30	e	4.83 ± 0.57	3.33 ± 0.62 (68.33%)	1.08 ± 0.32 (25.67%)	0.34 ± 0.15 (7.00%)	0.02 + 0.04 (0.33%)
	c	10.42 ± 4.69[a]	8.35 ± 3.64[a] (80.83%)	1.67 ± 1.02 (16.00%)	0.30 ± 0.19 (2.40%)	0.00 ± 0.00 (0.00%)
60	e	4.05 ± 0.98	3.04 ± 1.05 (73.67%)	0.66 ± 0.25 (17.00%)	0.34 ± 0.05 (9.00%)	0.01 ± 0.03 (0.33%)
	c	7.57 ± 1.40[c]	6.02 ± 1.18[b] (80.67%)	1.21 ± 0.37[a] (15.83%)	0.08 ± 0.14[b] (2.83%)	0.06 ± 0.1 (0.67%)
90	e	3.20 ± 0.41	2.36 ± 0.28 (73.83%)	0.58 ± 0.16 (17.40%)	0.29 ± 0.07 (9.00%)	0.01 ± 0.01 (0.17%)
	c	4.90 ± 0.48[b]	3.64 ± 0.38[b] (74.25%)	0.96 ± 0.12[b] (19.50%)	0.15 ± 0.11 (3.17%)	0.03 ± 0.03 (0.50%)
120	e	5.57 ± 1.05	4.33 ± 0.97 (77.33%)	0.69 ± 0.27 (12.67%)	0.56 ± 0.12 (10.00%)	0.00 ± 0.00 (0.00%)
	c	3.83 ± 1.14	2.98 ± 1.18 (76.50%)	0.61 ± 0.32 (16.83%)	0.19 ± 0.02 (6.30%)	0.00 ± 0.00 (0.00%)

e - experimental animal; c - control animal (non-infected); a - $P < 0.05$, b - $P < 0.01$, c - $P < 0.001$

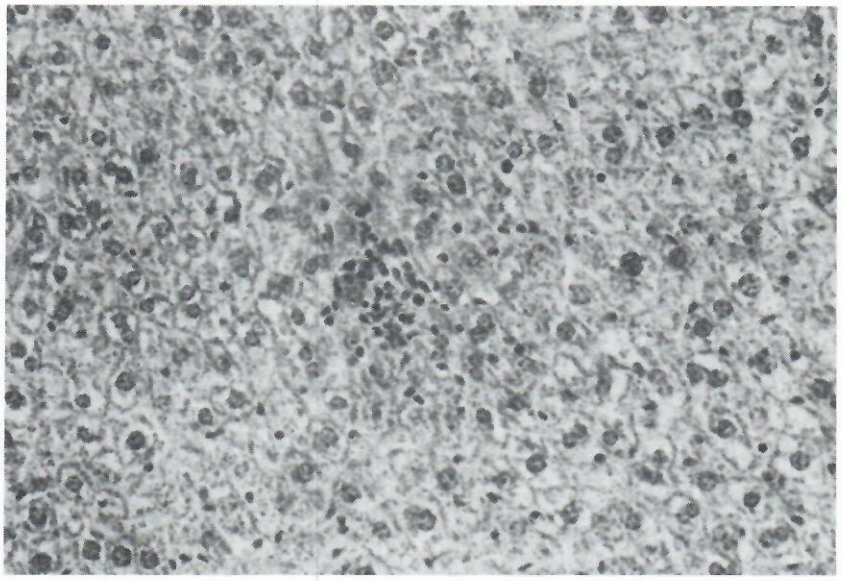

Figure 1. Liver. Accumulation of lymphocytes and macrophages in sinusoidal places. HE (x 32)

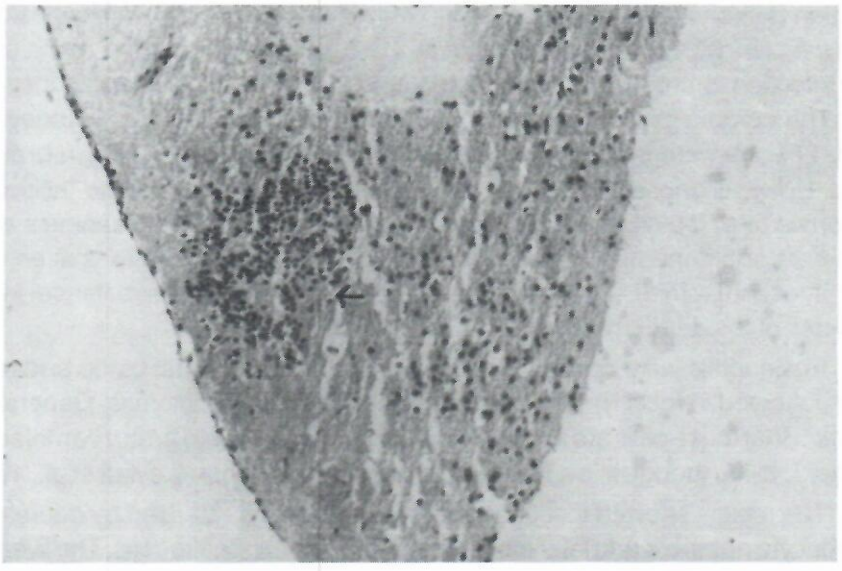

Figure 2. Brain. Focal infiltration of lymphocytes in the gray matter (Left). HE (x 160)

DISCUSSION

The results demonstrated that the number of peripheral blood leukocytes, including lymphocytes, was decreased already at days 10 in the experimental animals. On the other hand, the number of professional phagocytic cells, that is neutrophils and monocytes, increased in the second half of the experiment.

Studies in animals have revealed that T-cells are fundamental to the control of parasite multiplication. A decrease of lymphocytes in the peripheral blood of *E. cuniculi* infected animals could be induced by increased dying of these cells after interaction with macrophages and their movement into tissues and later participation in focal tissue infiltration. This is supported by the statement that after an initial phase of activation and differentiation to effector cells, most activated peripheral T-cells die upon further encounter with antigen (Kruisbeek and Amsen, 1996).

The significant increase of monocytes in peripheral blood found in *E. cuniculi* infected animals is a sign of the activation of macrophages. This activation of macrophages is a general feature of the early stage of parasitic infection. These cells constitute the main source of tumor necrosis factor (TNF-α) and interleukins in organisms (Tracey and Cerami, 1992).

Similarly, the recent work by Didier and Shadduck (1994) demonstrated the important role of macrophages for controlling replication of the microsporidia.

The finding of lymphocytic infiltration in liver sinuses at 10 days post infection demonstrates that focal non-purulent hepatitis is the earliest lesion in mice infected with *E. cuniculi.* Cox et al. (1979) observed that the acute stage post infection is characterized by formation of lesions in the lung, kidney and liver. The chronic form is typfied by microgranulomas in the brain, kidney and heart. Lesions were seen in the second half of our experiment also in brain ad heart. However these lesions only consisted of focal lymphocytic infiltration. Pastorova et al. (1997) found changes in the neuroendocrine messanger system in animals spontaneously infected with *E. cuniculi.* The brain lesions seen early after infection with *E. cuniculi* suggest the possibility of misbalance in the products of the endocrine system.

Eosinophils were not increased in numbers in peripheral blood and rarely were observed in focal lymphocytic infiltration or microgranulomas. Generally, it is known that these cells are not the dominant cell response to protozoan infection (Bignold, 1995) though it is a T-cell dependent phenomenon (Levkut et al., 1995).

The results showed that changes of WBC are characterised by decreasing lymphocyte numbers and later increasing of blood phagocytic cells. The liver was confirmed to be the first infected organ. However focal non-purulent inflammation

was seen also in lung, kidney, brain, and heart. It may be supposed that some relationship exists between changes in WBC numbers and movement of these cells into infected tissues.

Acknowledgement

This work was supported by Slovak Grant committee Nos. 4223/97 and 4358/97

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PROMENE U BROJU BELIH KRVNIH ĆELIJA I FORMIRANJE LEZIJA KOD MIŠEVA EKSPERIMENTALNO INFICIRANIH SA E. CUNICULII

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SADRŽAJ

Broj belih krvnih ćelija (WBC) i formiranje histoloških lezija ispitivano je kod miševa eksperimentalno inficiranih E. cuniculi observacijom u periodu od 120 dana.

Utvrđeno je značajno opadanje broja nekih leukocita uključujući limfocite 10, 30, 60 i 90-og dana posle infekcije. S druge strane broj monocita povećan je u drugoj polovini eksperimenta. Prvo su se razvile iluzije u jetri a kasnije i na drugim proučavanim organima (mozak, pluća, bubreg i srce). Zakqučeno je da postoji veza izmedu promena u broju WBC i pokretqivosti ovih ćelija u inficiranom tkivu.