[Jap. J. Parasit., Vol. 31, No. 3, 203-210, June, 1982]

Clinico-pathological changes of Mice Following Primary and Secondary Infections with *Hymenolepis nana* Eggs

1. Changes in Hematological Values

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(Received for publication; April 8, 1982)

Key words: hematology, infection, *Hymenolepis nana*, mice, anemia, eosinophilia

Introduction

The infection of *Hymenolepis nana* is often observed to occur in man, particularly in children, and conventional laboratory mice are known to be frequently infected with this parasite. In light infections, the symptoms are usually absent, or are limited to vague abdominal discomfort. The patients with heavy infections, however, sometimes complain of abdominal pain, vomiting, weight loss, diarrhea, and other clinical signs (Thomas, 1964).

It has been shown that *H. nana* is strongly immunogenic. There are many reports on the acquired immunity to *H. nana* infection in mice. These include results of infection experiments (Hearin, 1941; Heyneman, 1962 a,b), histopathological observations of the infection site of the larvae (Bailey, 1951; Friedberg *et al.*, 1979; Miyazato *et al.*, 1977; Miyazato *et al.*, 1979; Furukawa *et al.*, 1981), and results of immunochemical analysis (Di Conza, 1969; Ito, 1975). These results suggest that the patients with *H. nana* infections may have various parenteral or local pathologic changes caused by the elicitation of protective immunity. There is yet few study on the clinico-pathological changes due to infection of *H. nana*. Such a study should contribute fundamental information concerning human hymenolepiasis. A detailed study on this aspect in mice is also important because mice are currently used as experimental animals.

H. nana larvae derived from primary infection live in the intestinal villi of mice for only 4 days before leaving into the intestinal lumen, and infected mice acquire a strong protective immunity within short period of time (Heyneman, 1962 b). In addition, the elimination of challenge larvae from the intestinal villi of immunized mice seems to be completed during 2 days after secondary infection (Friedberg *et al.*, 1979; Miyazato *et al.*, 1979; Furukawa *et al.*, 1981). Accordingly, we studied hematological and clinico-chemical studies in mice following primary and secondary infections with *H. nana* eggs, and described the results of the hematological examinations in the present report.

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Materials and Methods

Animals: Male and female ddY mice used in the present study came from a colony maintained in our laboratory for several years. They were determined by egg floatation examinations to be free of *H. nana* infection. The mice had free access

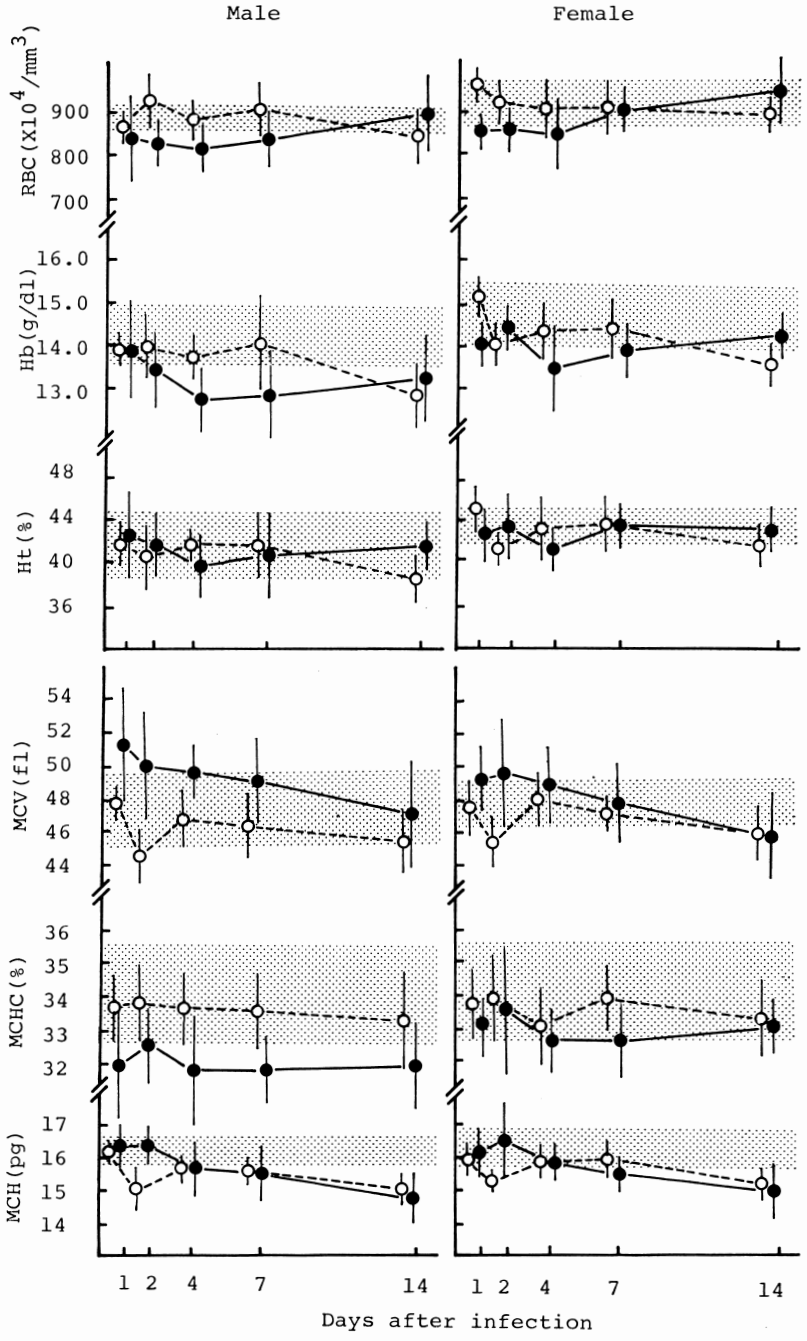

Fig. 1 Hematological changes following infection with *H. nana* eggs in male (*left*) and female (right) mice. ○·····○; primary infection, ●——●; secondary infection. Dotted areas indicate the range (mean ± SD) of uninfected control mice.

to diet and water. All the mice were 6 weeks old and weighed 32±3 g in male and 25±2 g in female mice at the beginning of the experiment.

Preparation and administration of *H. nana* eggs: Adult worms of *H. nana* were obtained from previously infected ddY mice. Eggs were teased from gravid proglottids of adult worms, rinsed and suspensed in physiological saline. Egg shells were removed by stirring the egg suspension with glass beads just before infection. A 0.1 ml of suspension containing desired number of eggs was administered directly into the stomach of mice using a syringe fitted with a blunt 18 gauge hypodermic needle.

Experimental design: Three groups of mice were examined. Group 1 (Primary infection group) comprised 60 mice in each sex. They were infected orally with 1000 eggs. Twelve mice in each sex were exsanguinated 1, 2, 4, 7 and 14 days after infection respectively and their blood samples were submitted to hematological examinations. The small intestines of 12 mice killed 4 days after infection were examined for cysticercoid infections by the method of Hunninen (1935). They had an average of 128 intestinal cysticercoids (range 8-341). Group 2 (Secondary infection group) comprised 60 mice in each sex. They were initially exposed to 1000 eggs, and then challenged with 2000 eggs 14 days after primary infection. In group 3 (Control group) 12 mice in each sex remained uninfected and were exsanguinated between 6 and 8 weeks after birth.

Prior to blood collection, mice were deprived of food for 12 hours. Blood was collected from the postcaval vein of mice under light anesthesia with sodium pentobarbital (25.6 mg/kg), and transferred into the sampling bottle containing anticoagulant (Toa Electronics Ltd, Tokyo, Japan). Blood sampling was carried out between 8:00 A.M. and 11:00 A.M. to avoid possible errors due to the diurnal fluctuations of the values to be measured.

Hematological examination: An electric cell counter (Toa Electronics) was used for red and white blood cell (RBC and WBC) counting. Hemoglobin (Hb) was determined by cyanmethemoglobin method. Hematocrit (Ht) was determined manually with heparinized capillary tubes. For differential leukocyte counts, thin blood smears were stained with Giemsa and number of monocytes, lymphocytes, neutrophils, and eosinophils were counted under light microscope. Wintrobe corpuscular constants including mean corpuscular hemoglobin (M CH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV) were calculated from the data of RBC, Hb, and Ht. The statistical significance of differences between control group and experimental one were examined by Student's *t*-test. The data were expressed by mean±standard deviation of the mean.

Results

Changes of RBC counts, Hb, Ht, MCV, MCHC, and MCH values are shown in Fig. 1. Total and differential WBC counts are shown in Figs. 2 and 3, respectively.

Almost all of the values concerning red blood cells of experimental group after primary infection closely followed those of control animals. There was, however, an indication that primary infection group had a tendency to a slight anemia, which was evidenced by a slight decrease of Hb, Ht, and MCH values in both male and female mice especially at 14 days after primary infection. The occurrence of anemia in both male and female mice became clear after secondary infection, as evidenced by significant decrease of RBC count, Hb, MCHC, and MCH values.

Total and differential WBC counts following primary infection remained within

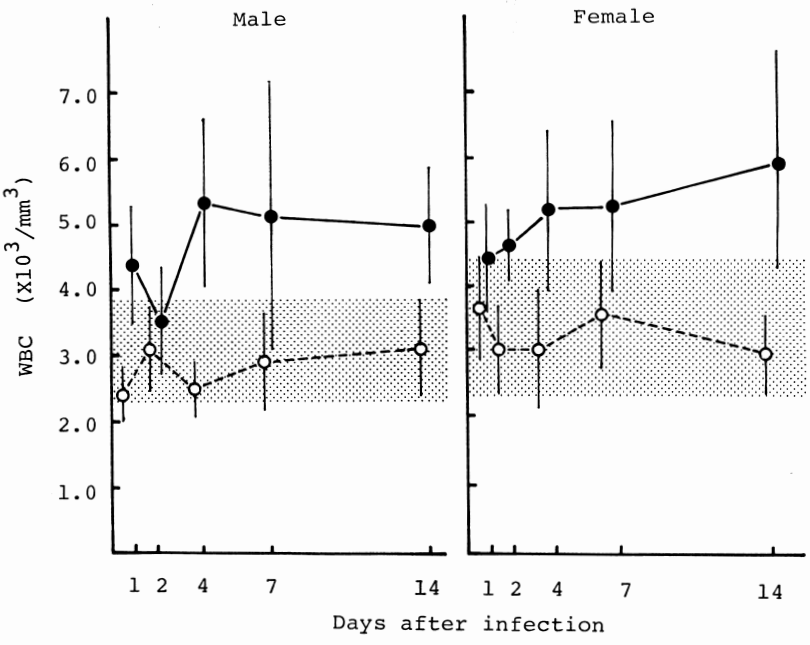

Fig. 2 Changes of total WBC counts following infection with *H. nana* eggs in male (left) and female (right) mice. ○·····○; primary infection, ●——●; secondary infection. Dotted areas indicate the range (mean ± SD) of uninfected control mice.

their normal ranges throughout the course of experiment. The total WBC count increased abruptly after secondary infection in both sexes and the higher values continued for 14 days. There was an increase in eosinophil count after secondary infection. Eosinophilia in male mice was observed as early as 1 day after secondary infection, peaked at Day 7 and gradually declined during the next 1 week. In female mice, an apparent eosinophilia was observed at Day 4 after secondary infection, peaked at 7th day and gradually declined thereafter. A transient decrease in lymphocytes with simultaneous increase of neutrophils was observed only in male mice immediately after of secondary infection. No significant change was showed in the value of monocytes all the course of secondary infection.

Discussion

Erythrocyte values showed that there was a slight decrease in Hb and MCH values after primary and secondary infections, suggesting that the infected mice were slightly anemic. The decrease of RBC count, Hb, MCHC, and MCH values were more prominent in male mice after secondary infection. Anemia is not a common clinical feature of cysticercosis caused by various taeniid cestodes in man and domestic animals (Ansari and Williams, 1976). Our observations, however, indicated that the infection of laboratory mice with *H. nana* eggs led to slight anemia, which became more prominent after secondary infection. These observations appear to be compatible with the fact that the intestinal villi of mice are damaged by the invasion

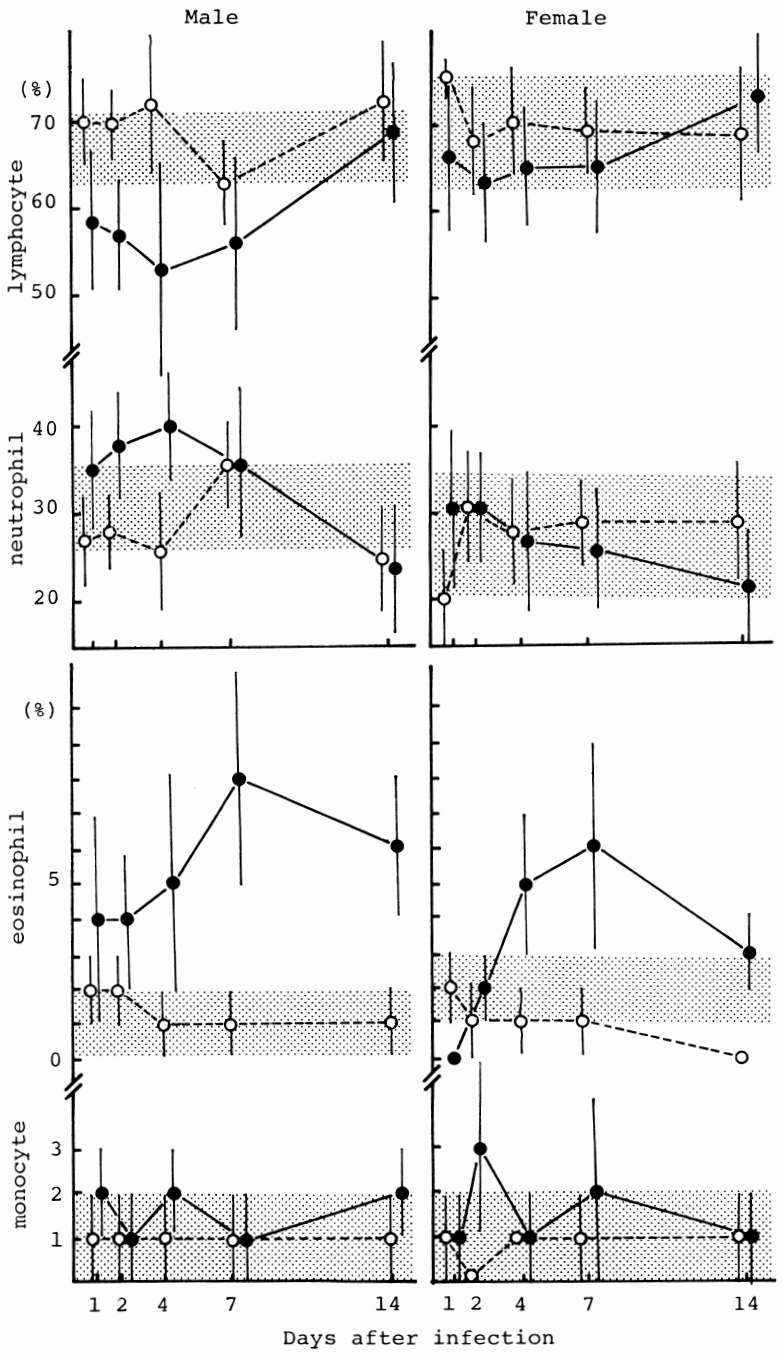

Fig. 3 Changes of differential WBC counts following infection with *H. nana* eggs in male (left) and female (right) mice. ○·····○; primary infection, ●——●; secondary infection. Dotted areas indicate the range (mean ± SD) of uninfected control mice.

of *H. nana*, and that the histological intestinal damage was more apparent in immunized and challenged mice (Friedberg *et al.*, 1979; Miyazato *et al.*, 1979). It is probable therefore that malnutrition or malabsorption due to histological damage of small intestine may cause a slight anemia in infected mice. The result of clinico-chemical examination of mice infected with *H. nana* (Shimoda *et al.*, manuscript in preparation) also showed that the infected mice had a hypoproteinemia, probably due to unnourishment.

Total and differential WBC counts after primary infection were similar to those in control animals during 14 days after infection. In contrast, total WBC counts increased abruptly on the 1st day of secondary infection. The higher values continued for 14 days after infection. The higher WBC counts reflected relative increase in circulating eosinophils. Eosinophilia was first observed as early as 1 day after secondary infection in male mice, peaked at Day 7 and gradually declined for the next 1 week. The same pattern of undulated eosinophilia was observed in female mice, though the relative values was slightly lower than that of male mice. It was evident therefore that secondary infection with *H. nana* eggs elicited an apparent peripheral eosinophilia, although the absolute eosinophil counts was not made in the present study.

Eosinophilia has been reported in various helminthiasis and several authors have speculated that eosinophil has a parasiticidal function with or without cooperation of antibody and complement (Reviewed by Butterworth, 1977). Ansari and Williams (1976) and Ansari *et al.* (1976) have demonstrated that rats infected with *Taenia taeniaeformis* eggs had a apparent primary and secondary peripheral eosinophilia with the local accumulation of eosinophils at the infection site. They suggested that immunologically mediated chemotaxis or some chemotactic substances produced by developing cysticercus could be responsible for the local accumulation of eosinophils, and that the sustained eosinophilia in peripheral blood must come as a result of increased bone marrow production. The exact role of eosinophils in eliminating challenge larvae of *H. nana* is still uncertain. But there are several reports on the local accumulation of eosinophils around the oncosphere larvae of *H. nana* in immunized mice (Bailey, 1951; Friedberg *et al.*, 1979; Miyazato *et al.*, 1979). Recently, Furukawa *et al.* (1981) reported the accumulation and attachment of eosinophils and macrophages onto the surface of challenge larvae in immunized mice. Accordingly, a peripheral eosinophilia after secondary infection observed in the present study might reflect local response of eosinophils around the challenge larvae.

The transient decrease in the relative value of lymphocyte with simultaneous increase of neutrophils observed solely in male mice at an early stage of secondary infection is hard to explain. Circumstantial evidence shows that lymphocytes are closely related to the initiation of protective immunity and anamnestic response against *H. nana* infection in mice (Okamoto, 1968; Okamoto and Koizumi, 1972). It is expected therefore that some immune mechanisms are related to a transient lymphopenia. However, this phenomenon in male mice requires further investigation. At present the role of neutrophils and monocytes in *H. nana* infection is not known.

The present report concerned with a part of a series of clinico-pathological study on *H. nana* infection in mice. In the following papers we will report the results of clinico-chemical examination on *H. nana* infection in mice.

Summary

The changes of hematological values of male and female ddY mice following

primary and secondary infections with *Hymenolepis nana* eggs were studied. There was a slight anemia in infected mice, as evidenced by a slight decrease of Hb and MCH values after either primary or secondary infection. Total and differential WBC counts following primary infection closely followed those in control animals. In secondary infection, total leukocyte count and percentage of neutrophil increased abruptly at day 1 and higher values continued for 14 days after infection. The transient decrease in the relative values of lymphocyte with simultaneous increase of neutrophils observed only in male mice at an early stage of secondary infection.

Acknowledgments

Excellent technical assistance of Miss K. Mizokawa, Miss K. Takada and Miss M. Yamano are greatly acknowledged.

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小形条虫の初感染および再感染時におけるマウスの臨床病理学的検討

1. 血液学的所見

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マウスに実験的に小形条虫卵を経口感染させ，初感染および再感染の初期における宿主の血液学的変化について検討した．

初感染，再感染のいずれの場合も，ヘモグロビン濃度，平均赤血球ヘモグロビン値のわずかな減少がみられることから，小形条虫に感染したマウスでは軽度ではあるが貧血がおこることが明らかとなった．貧血は初感染より再感染の場合により強く認められた．これは多数感染による栄養物吸収不良に基づく栄養障害性貧血であろうと思われる．初感染群では白血球分類比は対照群と差はなかった．これに対して，再感染群では感染直後から白血球数増加，好酸球比率の増加がみられ，少なくとも感染後14日目まで高値のままであった．再感染初期に好中球比率の増加とリンパ球比率の相対的な減少が雄マウスにおいてのみみられた．

以上のことから，小形条虫卵の感染を受けたマウスでは軽度ではあるが，貧血，白血球数増多，好酸球比率の増加などの変化がおこることが明らかとなった．