[Jpn. J. Parasitol., Vol. 36, No. 6, 375–382, December, 1987]

Kinetics of Erythroblast and Eosinophil Production in the Bone Marrow of the Mouse Infected with *Schistosoma japonicum*

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(Received for publication; June 25, 1987)

Abstract

Hematological changes in the peripheral blood and the bone marrow of mice infected with *Schistosoma japonicum* were examined. The cellular kinetics of erythroblasts and eosinophils in the bone marrow were observed under an electron microscope. Hypoplasia of erythroblasts was observed in the bone marrow of infected mice. Erythroblast production in the bone marrow began to decrease in the 6th week. Erythroblast percentages decreased to 1.8% and 3.0%, respectively in two infected mice, whereas the value in the control was restored to 16.0% in the 12th week. The number of RBC in the peripheral blood of infected mice decreased to $4.5 \times 10^6/mm^3$ in the 12th week, though the control value kept a normal level. The anemia in mice was normocytic. Serum Fe concentration did not show any difference between the infected and the control mice in the 6th week when production of erythroblasts began to decrease in the bone marrow. Eosinophil percentages in the bone marrow rose from the 6th week after the infection and reached 18.9% and 17.5%, respectively in two infected mice in contrast with 5.0% in age-matched control mouse in the 12th week. When eosinophilopoiesis was enhanced by the infection, cluster formation of eosinophils was observed in the bone marrow.

Key words: *Schistosoma japonicum*, Bone Marrow, Eosinophil, Anemia, Erythroblast, Electron Microscopy

Introduction

Both men and animals infected with *Schistosoma japonicum* show severe anemia in the acute stage. It was considered that anemia was caused by the intake of erythrocytes as a nutrient of the worms, bleeding from intestinal wall or hemolysis (Mahmoud and Woodruff, 1972). In addition to anemia, eosinophilia in the peripheral blood is a prominent feature especially in the acute stage of schistosome infection. The mechanism of eosinophil kinetics has attracted much interest recently. Mahmoud *et al.* (1975) reported an increase of eosinophil production in the mouse bone marrow following formation of egg granulomas in the liver, after the eggs of *Schistosoma mansoni* were injected intravenously. Ishii *et al.* (1981) recognized no colony stimulating factor activity in soluble egg antigen and adult worm antigen of *S. japonicum* when bone marrow cells were cultured *in vitro*. These findings suggest that eosinophilopoiesis is regulated by complicated mechanisms.

For the purpose of clarifying the microenvironmental situation of erythropoiesis and eosinophilopoiesis in the bone marrow, we examined the kinetics of erythroblast and eosinophil formation in the bone marrow of the mouse infected with *S. japonicum* by electron microscopy.

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This study was financially supported by a grant from Ohyama Health Foundation, Tokyo.

Materials and Methods

Animals

Four-week-old male ddY mice obtained

from Kitayama LABES Co., Ltd. (Kyoto, Japan) were intraperitoneally infected with 60 cercariae of *Schistosoma japonicum* (Kofu strain).

Hematological examination

After the infection, mice were killed with chloroform at the 1st, 2nd, 3rd, 6th, 9th, 12th, 15th and 25th week. The blood was obtained by cardiac puncture and examined for RBC, WBC, Hb, Ht and mean corpuscular volumes (MCV) using an automatic blood cell counter (Toa Co. Ltd., CC-800). Data were obtained from 3 animals each in experimental groups and 2 in age-matched control groups, and shown as mean values. These measurements during 6 weeks after the infection were repeated twice. The eosinophils in the peripheral blood were counted on blood smears stained with May-Grünwald-Giemsa solution.

Serum Fe concentrations of four mice each in the control and experimental groups were measured by Nitroso-PSAP direct method of FeC Wako Kit (Wako Pure Chem. Indust., Tokyo) in the 6th, 10th and 14th week.

Electron microscopy

The bone marrow was removed from right and left femurs and immediately put into the Karnovsky's glutaraldehyde and paraformaldehyde mixture. The bones were cut at the middle portion and fixed for 20 hours at 4°C. The bone marrow was removed from the bone by a thin blade and post-fixed with 2% OsO_4 for 2 hr after washing 5 times with 0.1 M cacodylate buffer (pH 7.4) with 4.5% sucrose added to adjust osmotic pressure. The bone marrow specimens were dehydrated and embedded in a mixture of Epon 812 and 815 resin. The bone marrow was sectioned on a Porter-Blum Ultramicrotome, stained with uranyl acetate and lead hydroxide and examined by a Hitachi HS-8 transmission electron microscope.

Counts of eosinophil and erythroblast in the bone marrow

Identification of eosinophil and erythroblast in the bone marrow sections was carried out according to the criteria of Breton-Gorius and Royes (1976) and Miwa (1983). As an ultrastructural feature, eosinophils in promyelocyte stage already have characteristic crystalloid bodies in some large granules. Mature erythroblasts (polychromatic I, II) are less abundant with organelles in cytoplasm. The nucleus is eccentric and has a pycnotic appearance with large dense clumps of chromatin. Pictures of a large area of the bone marrow were taken with 2400 magnification and reconstructed by the means of the photographic prints to examine the rates of eosinophils with core granules and of mature erythroblasts in 150–300 nucleated cells. Eosinophil and erythroblast rates were shown as a mean value from at least three regions in the bone marrow. The bone marrows of two mice in three experimental mice and a mouse in two age-matched control mice were examined in each stage, respectively.

ELISA

Antibody titers in mice sera to soluble egg antigen (SEA) of *S. japonicum* prepared according to the method described by Ishii *et al.* (1982) were examined by means of an ELISA which was described by Matsuda *et al.* (1984). Horse-radish peroxidase labeled anti-mouse Ig (Dakopatts, Co., France) was used as a secondary antibody. Optical density at 405 nm was measured with a EIA reader EL-307 (Bio-Tek Instruments, Inc., U.S.A.).

Results

Hematological changes

Changes in the number of RBC after the challenge with *S. japonicum* cercariae are shown in Fig. 1. The mean value of RBC was about $6.3 \times 10^6/mm^3$ before infection. The number of RBC increased to about $9 \times 10^6/mm^3$ in the 2nd week in both control and experimental groups. In the control group, the values maintained similar level from the 3rd week to the 15th week whereas the values in the experimental group decreased gradually from the 6th week, and became down to $4.5 \times 10^6/mm^3$ in the 12th week which was about half of the control level. Mice became anemic at

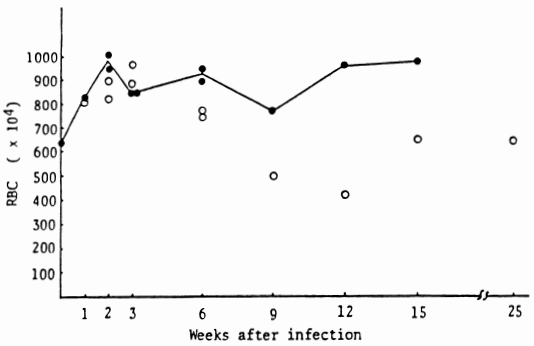


Fig. 1 Changes in the number of RBC in the peripheral blood. Blood collection at the 25th week failed in the control mouse. Each dot was shown as a mean value of two mice (control) and three mice (experiment).
(● control, ○ experiment)

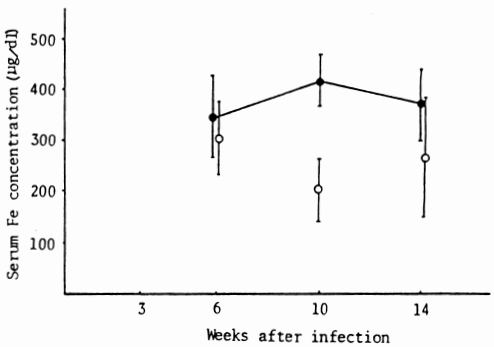


Fig. 2 Changes in serum Fe concentration. There are no significant difference between the experimental and the control groups in the 6th week at the stage appearing anemia. However, in the 10th week, serum Fe concentration of experimental groups decreased significantly. Vertical bars show SD.
(● control, ○ experiment)

this stage and thereafter anemia subsided gradually. Changes of Ht and Hb were very similar to those of RBC. MCV was slightly higher in the control (45.3 μm^3) than in the experimental groups (42.3 μm^3) in the 6th week. Though serum Fe concentration showed lower values (301 μg/100 ml) in the experimental group than the control (344 μg/100 ml) in the 6th week, significant differences could not be seen between the control and experimental groups. However, the difference in serum Fe concentrations was substantially low in the 10th week (Fig. 2).

The number of WBC began to increase in the 6th week contrary to the changes in RBC and showed a peak of $3.8 \times 10^4/mm^3$ while that of the control group was $5.5 \times 10^3/mm^3$ in the 12th week. The percentage of eosinophils in the peripheral blood rose to 8–15% in the 3rd week and maintained that level thereafter.

Observation by an electron microscope

In the bone marrow, the percentages of eosinophils increased and those of mature erythroblasts decreased at the early stage in both control and experimental groups. The percentages of eosinophils rose in the 1st and 2nd week, and restored to the normal value in the 3rd week. The percentages of erythroblasts decreased drastically until the 3rd week in both groups, but there was a marked difference starting from the 6th week.

As shown in Fig. 3, the percentage of eosinophils with core granules began to increase in the 6th week in the bone marrow. In the 12th week, the percentages of eosinophils were 18.9% and 17.5%, respectively in two infected mice, in comparison with 5.0% in age-matched control mouse. The percentages of eosinophils were still high (12.0% and 10.3%) in the 15th week, and became near to normal in the 25th

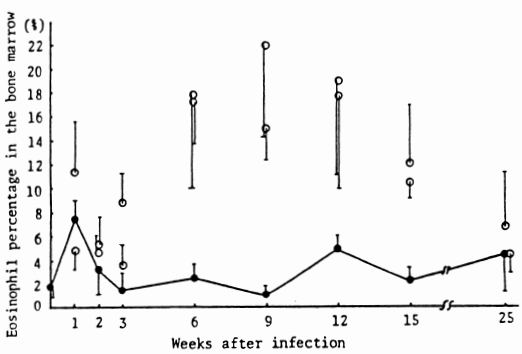


Fig. 3 Changes in percentage of eosinophils (with core granules) to nucleated cells in the bone marrow. Each dot is shown as the mean value of three different areas at the middle portion of the bone marrow. Vertical bars show SD.
(● control, ○ experiment)

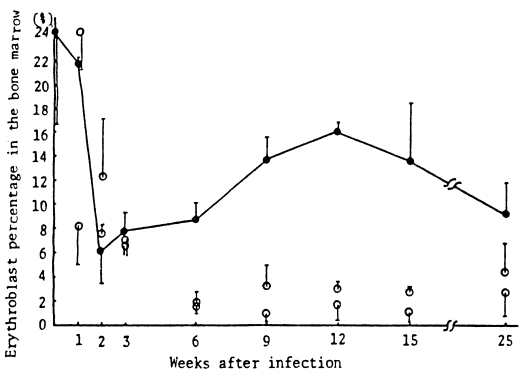


Fig. 4 Changes in percentage of mature erythroblast to nucleated cells in the bone marrow. Each not is shown as the mean value of three different areas at the middle portion of the bone marrow. Vertical bars show SD.
(• control, ○ experiment)

week.

The percentages of erythroblasts to the nucleated cells in the bone marrow decreased to 1.7% and 1.9%, respectively in two infected mice whereas the value in the control restored to 8.7% in the 6th week (Fig. 4). Though the percentages of erythroblasts in the 12th week showed similar low values (1.8% and 3.0%) to those in the 6th week in infected mice, that in a control mouse was restored to 16.0%, which was within a normal range. The production of erythroblasts was still low and a restoration could not be seen in the bone marrow of the experimental groups even in the 25th week.

Photo 1 shows a part of the bone marrow of an infected mouse in the 15th week after the infection. The bone marrow is occupied by many eosinophils with core granules. One eosinophil during mitosis stage is seen in the middle part of this field. Most of the other cells are polymorphonuclear cells, and have small electron-dense granules and abundant well-developed rough endoplasmic reticulum in the cytoplasm. These cells are neutrophils. Though erythroblasts could not be seen in this photograph, the percentage of total erythroblasts in this bone marrow was 2.9%. The percentage of eosinophils was 18.2%. Contrary to these findings, the control as shown in Photo 2 showed only a few eosinophils (1.0%) and numerous erythroblasts (19.4%).

The microenvironmental situation of eosinophil and erythroblast production in the bone marrow was observed under an electron microscope. It is known that erythroblastic islands exist near macrophages. Though we could not see a typical feature of islands in the control and experimental bone marrows, there was a tendency of two or three erythroblasts being adjacent to each other. Eosinophils seem to form clusters of two to eight eosinophils on EM picture.

ELISA

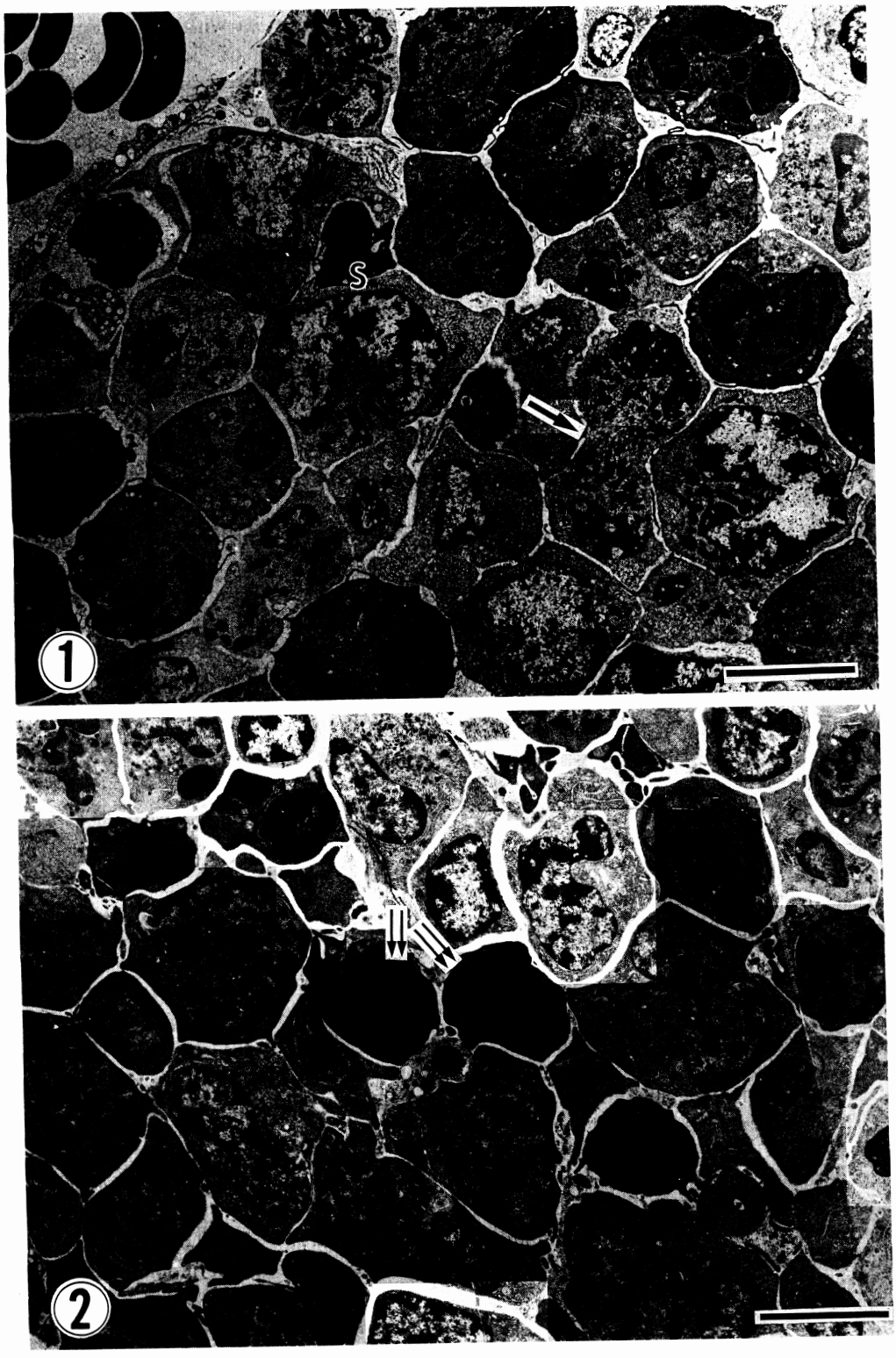
In the 6th week after the infection, the antibody to schistosome egg antigen started to raise although the egg granulomas in the livers were usually small; and by the 12th week this titer increased to more than 1:5120.

Discussion

It is generally considered that for the pathogenesis in schistosomiasis the deposition of eggs in the liver is more important factor than the burden of parasitic adult worms themselves (Warren 1963). Erickson (1965) reported that

Photo 1 A cross section of the bone marrow in the 15th week after the infection with *Schistosoma japonicum*. Many eosinophils form clusters near vascular sinus. A stromal cell (S) near eosinophil is noticed with a small and electron-dense nucleus, and dense cytoplasm. Most of all the neutrophils have a segmented nucleus and fine granules in the cytoplasm. In the lower right side of the photograph (arrow), eosinophil during mitosis can be seen. Eosinophils have already granules with cores at a promyelocyte stage in its developmental process and still possess an ability of mitosis. Bar indicates 5 μm.

Photo 2 A cross section of the bone marrow of an age-matched control mouse in the 15th week. In the central field, two erythroblasts (double arrows) are noticed. Neutrophils with a rod-, dumb-bell-shaped or segmented nucleus have fine granules in the cytoplasm and are adjacent to erythroblasts. Eosinophils are not shown in the photograph. Bar indicates 5 μm.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mice infected with *S. mansoni* cercariae irradiated with 3000 r to diminish oviposition, showed only slight anemia after 16 weeks of infection. In this experiment, mice appeared severely anemic (Fig. 1). The percentages of erythroblasts in the bone marrow decreased greatly and continuously after the 6th week as compared with the control group (Fig. 4). Experimental animals did not show significant differences in both serum Fe concentration (Fig. 2) and MCV to the control in the 6th week. It was considered that this anemia was an aplastic-anemia according to the Wintrobe's classification criteria of anemic diseases. The temporary decreases of erythroblast percentages in the 2nd week in both groups may be due to an alteration of keeping condition of mice, especially a water-supply system in our laboratory or Kitayama LABES. Mice appeared polycytemia which might be based on a hemoconcentration by a dehydration or other unknown causes at this period (Fig. 1). It is suggested that this polycytemia induces hypoplasia of erythroblast in the bone marrow (Filmanowicz and Gurney, 1961). These changes improved gradually thereafter in the control group.

Anemia in schistosomiasis is induced by several mechanisms: 1) intestinal bleeding, 2) decrease of red cell life span (da Silva *et al.*, 1963, Mahmoud and Woodruff, 1972), 3) hemodilution due to the high plasma volumes (Fiorillo *et al.*, 1954), 4) enhanced destruction of red cells in the spleen (Woodruff *et al.*, 1966) and 5) hemolysis by autoimmune reaction (Kurata, 1966). Kurata (1966) detected antibody to the nucleus and mitochondria of the liver cell in the serum of a rabbit infected with *S. japonicum*. He concluded that anemia was caused by hemolysis resulting from an autoimmunity due to the liver impairment by the egg deposition. In addition to these mechanisms, low production of erythrocyte in the bone marrow has been reported by Yoshimura in acute human schistosomiasis japonica (1952) and Hayashida in acute rabbit schistosomiasis japonica (1967). However, the observation of hypoplasia of erythroblasts in the bone marrow is not consistent with the findings in chronic human cases of schistosomiasis japonica. Hanazato (1958) reported that the bone marrow had conspicuously enlarged hematopoietic areas due to the increase of erythroblastic cells by proliferation. It might be due to differences between acute and chronic stages in schistosomiasis.

Eosinophilia in the peripheral blood did not show a prominent increase. However, the production of eosinophils in the bone marrow began to increase to 16–18% in the 6th week and this level was maintained thereafter (Fig. 3). McGarry (1983) reported that the eosinophil rate to the total WBC in the bone marrows of Ha/ICR mice infected with *S. mansoni* began to increase in the 6th week after infection, and became 44.3% in the 7th week. These value was higher than our values observed in ddY mice infected with *S. japonicum*. This discrepancy may be due to differences in the mouse strain and the parasite species. Eosinophil production in the bone marrow was also observed when mice were inoculated subcutaneously with SEA (50–100 μg protein) of *S. mansoni* with Freund's adjuvant (McGarry 1983). It seems necessary for high eosinophilopoiesis in the bone marrow that eggs retained in the liver act as a prolonged antigenic stimulus.

Basten and Beeson (1969) carried out detailed experiments to examine the roles of lymphocytes for an induction of eosinophilia. Evidences suggest that T lymphocytes or cell-mediated immunity is involved in the development of the granulomatous response to egg of schistosome (Weller and Goetzl, 1979, Chensue *et al.*, 1981, Weinstock and Boros, 1983). Antibodies to egg antigens were first detected in the 6th week. It suggests that immunological response is related to these manifestations of a hypoplasia of erythroblasts and an enhancement of eosinophil production in the bone marrow.

By electron microscopy, Sakai *et al.* (1981) showed an acute eosinophilopoiesis in the mouse bone marrow exposed to *Ascaris suum*. Eosinophils in the bone marrow increased to 5–45% and made clusters in it within 14 days

after a challenge. The characteristic feature was that branched stromal cells were present only in zones of marked eosinophilopoiesis. These results almost agree with our observation in the bone marrow of mice infected with *S. japonicum*. An electron microscopical study is more difficult and complicated than the method using a conventional light microscopy. However, we attempted to observe a real and intact arrangement of eosinophils or erythroblasts in the bone marrow. These cells could be identified clearly with an electron microscope. This method has a disadvantage in that an observation area is too small. Therefore, we counted the percentages of erythroblasts and eosinophils in three different areas of the bone marrow. There was not so great difference in the percentages of these cells in various regions of the bone marrow.

Hypoplasia of the erythroblasts and enhanced production of eosinophils were observed in murine schistosomiasis japonica. There are some evidences that anemia in schistosomiasis is, at least in part, of immunological origin (Mahmoud and Woodruff, 1972). Further research using cell-culture technique of the bone marrow should elucidate the relationship on productions of these cells.

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