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Eosinophil Responses in the Mice Infected with *Metagonimus yokogawai*

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Abstract

In mice infected with 100 metacercariae of *Metagonimus yokogawai,* worm expulsion began from day 10 of the primary infection and from day 3 of the secondary infection. Eosinophils in the blood increased to reach maximum level as about 2 times of those of control at day 7 of the primary infection. In the secondary infection, eosinophils more quickly and significantly increased than those in the primary infection. The results of worm recovery at day 10 of the primary infection or at day 3 of the secondary infection suggested that the kinetics of eosinophils might partly play a role to induce protective immunity for *M. yokogawai.*

Key words: *Metagonimus yokogawai,* mice, eosinophil responses

Introduction

Metagonimus yokogawai is an intestinal parasite distributing in Asian countries including Japan. Although the majority of human cases is subclinical, symptoms such as diarrhea and abdominal pain are occasionally seen in the heavy infections. Such clinical findings have been attributed to the pathological features in the small intestines of infected animals (Chai, 1979; Lee *et al.*, 1981; Kang *et al.*, 1983).

The author (1983) reported that *M. yokogawai* was expelled at day 10 to 14 of the infection and its expelling occurred more rapidly after reinfection in the infected mice. This finding was considered to be influenced by increase of intestinal permeability and mast cells in the small intestines of infected mice (Ohnishi and Taufan, 1984).

The present study dealt with worm recovery, total white blood cells (WBC) and eosinophils in the blood, intestine and bone marrow in the mice infected with *M. yokogawai.* The relationship between eosinophil responses and worm expulsion was discussed.

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Materials and Methods

Experimental schedule:

Six weeks old male ddY mice raised under specific pathogen free condition were infected orally with 100 metacercariae of *M. yokogawai* collected from fresh water fishes, *Tribolodon hakonensis.* All of the mice were kept in the conventional condition, each group of 10 infected mice was sacrificed at day 3, 5, 7, 10, 14 and 21 of the infection. Each group of the secondary infection given with 100 metacercariae at day 21 of the primary infection was similarly sacrificed at day 3, 5, 7, 10, 14 and 21. The age-matched 5 mice were provided as a control.

Worm recovery:

The recovery of worms was carried out according to the method of previous paper (Ohnishi, 1983). Small intestines were removed from carcasses, cut longitudinally and washed with tap water. After 3 hours, the worms were recovered from washing fluids.

Examinations of the blood, bone marrows and small intestines:

All of the mice anesthetized with ether were sacrificed by cardiac puncture. Blood samples were collected into 1.2% EDTA tubes. WBC and eosinophils in the blood were counted using Bürker-Türk type hemocytometer after

staining with Türk's and Hinkelmann's solution, respectively. The bone marrow cells (BMC) were collected from the femoral bones by washing with phosphate buffered saline containing 1.2% EDTA and the smears were stained with Giemsa's solution. The eosinophils including eosinophilic myelocytes and metamyelocytes were counted and expressed per 1,000 BMC as described by Ismail and Tanner (1972). The upper and middle parts of small intestines were cut out and opened longitudinally on the filter papers. The removed intestines were fixed by 10% formalin solution in physiological saline and the tissues embedded in paraffin were sectioned at 4 μm thick by a microtome. Sectioned specimens were stained with Giemsa's solution containing 1% eosin. Eosinophils per the 20 villus-crypt units (VCU) of small intestines were counted.

Statistical analysis:

The values in the primary and secondary infections were compared with those in the uninfected and primarily infected mice, respectively. A value of $P < 0.05$ by t- or non-parametric U-test was considered to be significant.

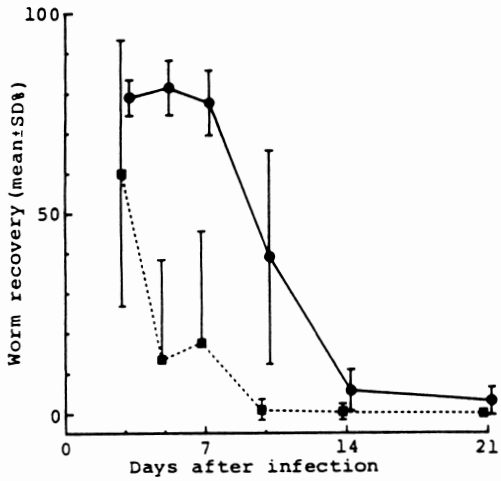

Fig. 1 Changes of worm recovery in the mice infected with *Metagonimus yokogawai*. The primary infection groups, ●—●; The secondary infection groups, ■- - - -■. All of the ddY mice were orally infected with 100 metacercariae. In the secondary infection, primed mice were orally challenged with 100 metacercariae at day 21 of the primary infection. Each value represents the mean ± SD of 10 infected mice.

Results

The rates of worms recovered from the intestines of infected mice were 77.7 ± 8.2%, 39.1 ± 26.8% and 5.7 ± 5.3% at day 7, 10 and 14 of the primary infection, and 60.1 ± 33.2% and 13.4 ± 25.3% at day 3 and 5 of the secondary

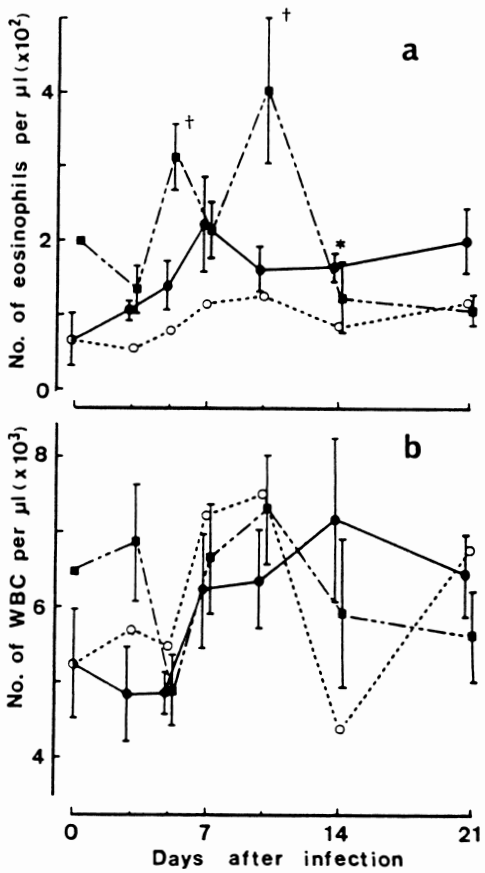

Fig. 2 Changes of eosinophils in the blood (a) and total white blood cells (WBC: b) of *M. yokogawai* infected mice. The primary infection groups, ●—●; The secondary infection groups, ■- – - -■; The uninfected groups, ○- - - - -○. Each value represents the mean ± SE of 10 infected and 5 uninfected mice. Significant difference ($p < 0.05$): *, the uninfected group versus the primary infection group; †, the primary infection group versus the secondary infection group.

infection, respectively (Fig. 1).

Eosinophils in the blood of primarily infected mice gradually increased to reach a peak of 222.0 ± 57.0/μl at day 7 and slightly decreased at day 10. Thereafter this level was continuously kept until day 21 (Fig. 2a). Eosinophil response observed in the secondary infection was earlier and higher than that in the primary infection. That is, eosinophils significantly increased at day 5 and 10 of the secondary infection though they slightly decreased at day 3 and 7. Somewhat increase of WBC was found from day 7 to 10 in the primary infection. WBC in the secondary infection significantly decreased at day 5 in comparison with that at day 3 (Fig. 2b).

Eosinophils in the blood significantly correlated with WBC in each of the infected mice at day 5 and 7 of the primary infection and at day 3, 5 and 10 of the secondary infection (r=0.708 to 0.753). There were significant but negative correlations between eosinophils in the blood and worm recovery in each of the infected mice at day 10 of the primary infection (n=10, r=−0.670, Y=−2.21X + 246, $P < 0.05$) and at day 3 of the seconary infection (n=9, r=−0.652, Y=−2.36X + 190, $P < 0.05$) (Fig. 3). However, eosinophils at day 5 of the secondary infection did not correlated with worm recovery (r=0.173).

Eosinophils per 1,000 BMC were 54.7 ± 16.5 ($P < 0.05$) at day 10 of the primary infection and 38.4 ± 12.3 at day 3 of the secondary infection (Fig. 4). At these times, eosinophils per VCU of the middle part of small intestine in the primary infection were much larger than those in

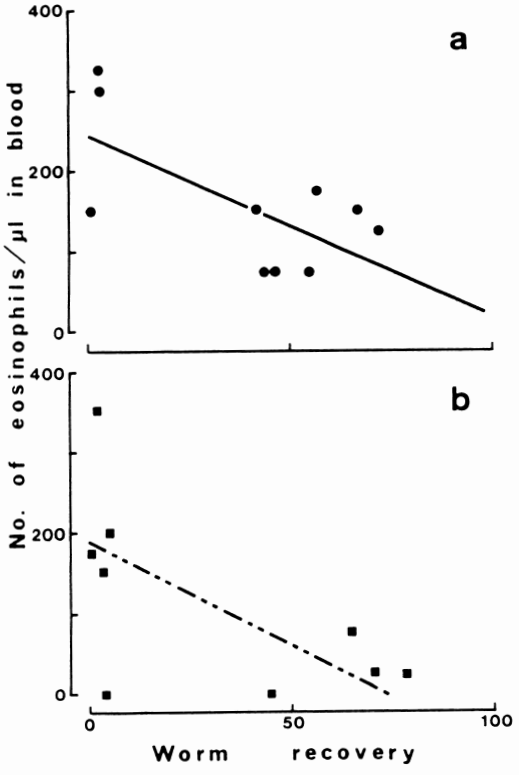

Fig. 3 Relations between eosinophils in the blood and worm recovery. (a) The primary infection group at day 10 (n=10, r=−0.670, Y=−2.21X + 246) and (b) The secondary infection group at day 3 (n=9, r=−0.652, Y=−2.36X + 190).

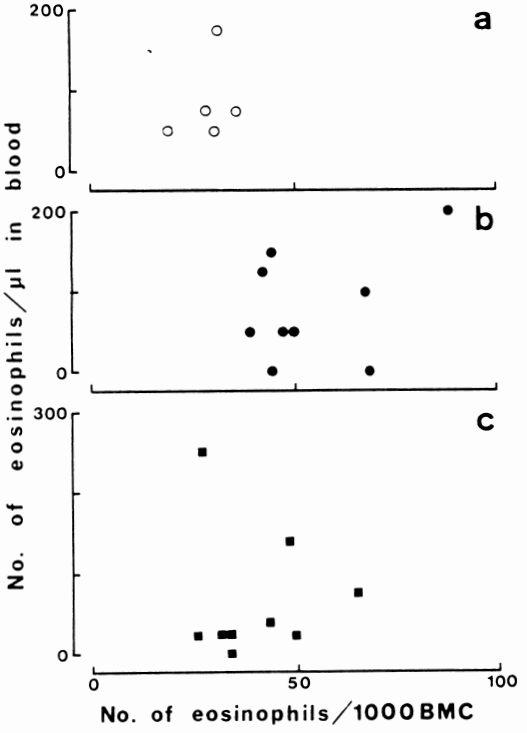

Fig. 4 Relations between eosinophils in the blood and those in the marrows of femoral bones. (a) The uninfected group (r=0.321). (b) The primary infection group at day 10 (r=0.397). (c) The secondary infection group at day 3 (r=−0.008).

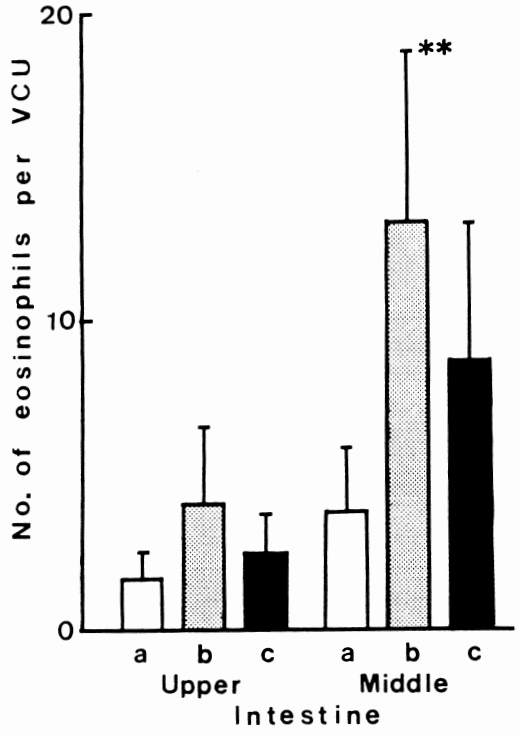

Fig. 5 Eosinophils in the upper and middle parts of small intestinal mucosae. (a) The uninfected groups. (b) The primary infection groups at day 10. (c) The secondary infection groups at day 3. **, Significant difference for the corresponding level of uninfected mice ($p < 0.001$).

the secondary infection (Fig. 5). No correlation was seen between eosinophils of the blood and those of the bone marrows or the small intestines.

Discussion

Increases of eosinophils in the blood of animals infected with various nematodes such as *Nippostrongylus brasiliensis* (Ogilvie *et al.*, 1978, 1980; Roth and Levy, 1980), *Trichinella spiralis* (Basten and Beeson, 1970; Basten *et al.*, 1970; Ismail and Tanner, 1972; Ogilvie *et al.*, 1980; Wakelin and Donachie, 1983) and *Strongylus ratti* (Moqbel, 1980) have been observed. In contrast, the present study of *M. yokogawai* did not demonstrated blood eosinophilia so much as those in the nematode infections. This difference may be attributed to species of parasites used, strain variation of the host (Ogilvie *et al.*, 1980; Wakelin and Donachie, 1983), infective dose of parasite (Ismail and Tanner, 1982) and the effective stimulus with larva migration (Despommier *et al.*, 1974). In the primary infection with *M. yokogawai*, eosinophils in the blood increased to reach about 2 times of those of control at day 7. As this time, the majority of the adult worms was present in the middle part of small intestine (Ohnishi, 1983). Therefore, the antigens from the worms may reflect to protective immunity. In addition, many cuticular spines of the worms may give the histological lesions inducing inflammation of the intestinal mucosa (Kang *et al.*, 1983). At day 10 that is the time of worm expulsion, eosinophils in the blood slightly decreased in spite of elevation of eosinophils in the bone marrow. The decrease of eosinophils in the blood cannot clearly be explained, however, numerous eosinophils were present in the middle part of small intestine. It is well known that eosinophil is mediated by immunological mediators derived from lymphocytes (Basten and Beeson, 1970) and neutrophils (Czarnetzki, 1978). Nawa and Hirashima (1984) reported that eosinophil chemotactic factors were produced by mesenteric lymph node cells at day 15 or 20 of the primary infection regulating tissue eosinophilia in *N. brasiliensis*-infected rats.

In the secondary infection with *M. yokogawai*, eosinophil responses in the blood were earlier and higher than those in the primary infection. However, eosinophils in the blood, bone marrow and intestinal mucosa at day 3 did not elevate so much as those of the primary infection. It seemed to be reasonable that eosinophils in the blood began to increase at day 3 and significantly elevated by day 5. Increase of eosinophils in the blood of secondary infection continued after diminishing antigenic stimulation with the worm of *M. yokogawai*. The kinetics of intestinal permeability and intestinal mast cells also seem to be of importance (Ohnishi and Taufan, 1984). The secondary infection might more strongly occur allergic re-

sponses than the primary infection.

On the other hand, the results of worm recovery at day 10 of the primary infection or at day 3 of the secondary infection suggested that the kinetics of eosinophils might partly play a role to induce protective immunity for *M. yokogawai.*

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References

1) Basten, A. and Beeson, P. B. (1970): Mechanism of eosinophilia. II. Role of the lymphocyte. J. Exp. Med., 131, 1288–1305.
2) Basten, A., Boyer, M. H. and Beeson, P. B. (1970): Mechanism of eosinophilia. I. Factors affecting the eosinophil response of rats to *Trichinella spiralis.* J. Exp. Med., 131, 1271–1287.
3) Chai, J.-Y. (1979): Study on *Metagonimus yokogawai* (Katsurada, 1912) in Korea. V. Intestinal pathology in experimentally infected albino rats. Seoul J. Med., 20, 104–117.
4) Czarnetzki, B. M. (1978): Eosinophil chemotactic factor release from neutrophils by *Nippostrongylus brasiliensis* larvae. Nature, 271, 553–554.
5) Despommier, D., Weisbroth, S. and Fass, C. (1974): Circulating eosinophils and trichinosis in the rat: The parasitic stage responsible for induction during infection. J. Parasitol., 60, 280–284.
6) Ismail, M. M. and Tanner, C. E. (1972): *Trichinella spiralis*: Peripheral blood, intestinal, and bone-marrow eosinophilia in rats and its relationship to the inoculating dose of larvae, antibody response and parasitism. Exp. Parasitol., 31, 262–272.
7) Kang, S.-Y., Cho, S.-Y., Chai, J.-Y., Lee, J.-B. and Jang, D.-H. (1983): A study on intestinal lesions of experimentally reinfected dogs with *Metagonimus yokogawai.* Korean J. Parasitol., 21, 58–73.
8) Lee, J. B., Chi, J. G., Lee, S. K. and Cho, S.-Y. (1981): Study on the pathology of metagonimiasis in experimentally infected cat intestine. Korean J. Parasitol., 19, 109–129.
9) Moqbel, R. (1980): Histopathological changes following primary, secondary and repeated infections of rats with *Strongylus ratti,* with special reference to tissue eosinophils. Parasite Immunol., 2, 11–27.
10) Nawa, Y. and Hirashima, M. (1984): Regulation of eosinophilia in rats infected with *Nippostrongylus brasiliensis.* 1. Eosinophil chemotactic factor produced spontaneously by mesenteric lymph node cells of infected rats. Int. Archs Allergy appl. Immun., 75, 264–269.
11) Ogilvie, B. M., Askenase, P. W. and Rose, M. E. (1980): Basophils and eosinophils in three strains of rats and in athymic (nude) rats following infection with nematodes *Nippostrongylus brasiliensis* or *Trichinella spiralis.* Immunology, 39, 385–389.
12) Ogilvie, B. M., Hesketh, P. M. and Rose, M. E. (1978): *Nippostrongylus brasiliensis*: Peripheral blood leukocyte response of rats, with special reference to basophils. Exp. Parasitol., 46, 20–30.
13) Ohnishi, Y. (1983): Experimental studies on *Metagonimus yokogawai* infection in the mouse. J. Juzen Med. Soc., 92, 585–597 (in Japanese).
14) Ohnishi, Y. and Taufan, M. (1984): Increase of permeability in the intestinal mucosa of mice infected with *Metagonimus yokogawai.* Jpn. J. Vet. Sci., 46, 885–887.
15) Roth, R. L. and Levy, D. A. (1980): *Nippostrongylus brasiliensis*: Peripheral leukocyte responses and correlation of basophils with blood histamine concentration during infection in rats. Exp. Parasitol., 50, 331–341.
16) Wakelin, D. and Donachie, A. M. (1983): Genetic control of eosinophilia. Mouse strain variation in response to antigens of parasite origin. Clin. Exp. Immunol., 51, 239–246.