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Kinetics of Goblet Cells and Mast Cells in the Intestine of C3H/HeN and BALB/c Mice Infected with *Echinostoma hortense*

Jee-Aee Im[1], Insik Kim[2], Yoon-Kyung Jo[3], Kyu-Jae Lee[4] and Yong-Suk Ryang[5†]

[1]Department of Laboratory Medicine, MizMedi Hospital, Seoul, Korea, [2]Department of Clinical Laboratory Science, School of Medicine, Eulji University, Daejeun, Korea 301-832, [3]Department of Clinical Pathology, Dongnam Health College, Suwon, Korea 440-714, [4]Depatment of Parasitology, Wonju College of Medicine, Yonsei University, Kangwon-do 220-701, Korea, [5]Department of Biomedical Laboratory Science and Institute of Health Science, College of Health Science, Yonsei University, Wonju, Korea 220-710

Mast cells and goblet cells have the ability to protect against parasites by increasing mucus production that traps and excludes worms and prevents their intimate contact with the gut mucosa in the host. In this study, we investigated the function of mast cells and goblet cells for the rejection of *Echinostoma hortense* (*E. hortense*). In addition, we used both C3H/HeN and BALB/c mice in order to examine whether mast cells and goblet cells function differentially according to the strains of mice. After an oral infection with 30 *E. hortense* metacercariae, the number of mucosal mast cells and goblet cells, as well as worm recovery rate, were observed in experimentally infected mice between 1 week and 8 weeks post-infection (PI). Worm recovery rates in C3H/HeN and BALB/c mice were 65.7% and 23%, respectively, in week 1 P.I., indicating that worm expulsion in C3H/HeN mice was higher than in BALB/c mice. Our results demonstrate that the period (week 3 P.I.) in which worm recovery falls rapidly is the same period that the number of goblet cells and mast cells reaches a peak. These results indicate that worm recovery significantly correlates with the growth rate of goblet cells and mast cells (P=0.0482). However, worm expulsion is not associated with goblet cells or mast cells in BALB/c mice.

Key Words: *Echinostoma hortense*, Goblet cell, Mast cell, Worm recovery rate, Mucosal immunity, C3H/HeN mice, BALB/c mice

INTRODUCTION

A continuous infection is not shown in a parasite-infected host because the infecting parasite are inhibited, destroyed and excreted by an immune response of the host. Many studies have reported about parasite expulsion, specifically the nematoda including *Trichinella spiralis*, *Nippostrongylus braziliensis*, *Strongyloides ratti* and trematoda including *Metagonimus yokogawai*, *Neodiplostomum seoulense*[3,16]. The mechanism of parasite excretion happens within weeks after infection and it is associated with T cell-dependent and T cell-independent mechanisms. Both IL-4 and IL-5 are produced, and antibodies are made in the B cells by a T cell-dependent mechanism. In addition, IL-3, IL-4, IL-9 and IL10 increase the growth of mucosal mast cells and goblet cells. After Ig E-stimulation, the mast cells secret histamine that increases permeability of intestinal epithelial cells and damages parasites. T cell-independent mechanisms stimulate the production of TNF-α, IL-1 and non-specific inflammatory molecules by the mast cells, proliferate goblet cells, and increase the production of mucosin that induces parasite expulsion. Mucosal mast cells, goblet cells, Ig A secretion and various cytokines increase parasite expulsion. There is a variety of immune response according to the kind of parasite and genetic background of the host[13,16]. Goblet cells play an important role in worm expulsion of *N. braziliens*, however, mast cells play an essential role in *S.*

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†Corresponding author: Yong-Suk Ryang, Department of Biomedical Laboratory Science, and Institute of Health Science, College of Health Science, Yonsei University, Wonju-city, Kangwon-do, Korea 220-710
Tel: 033-760-2422, Fax: 033-763-5224
e-mail: ryangys@eco.yonsei.ac.kr

ratti and *T. spiralis* infection[9,11,12,17]. Chai et al. (1993)[3] reported that the period in which worm expulsion in mice infected with *M. yokogavai* decreased markedly was in accordance with the period of the maximum growth of the mast cells. In rats infected with *N. seoulense* (*Fibricola seoulensis*), the worm recovery rate was associated with the growth of the mast cells[7].

In the present study, we investigated the relationship of worm expulsion and mast cells or goblet cells in C3H/HeN and BALB/c mice infected with *E. hortense*. In addition, we examined whether the worm recovery rate is associated with the strain of mice

MATERIALS AND METHODS

1. Metacercaria infection and worm recovery rate

BALB/c and C3H/HeN mice at 6 weeks old were obtained from the Korean Experimental Animal Center (Seoul, Korea). The experimental groups were divided into the control group and *E. hortense*-infected group, and each group includes five mice. Metacercariae of *E. hortense* were collected by an artificial digestion of *Misgurnus anguillicaudatus* caught in Munmak, Kangwondo. Thirty metacercariae were fed to each mouse via a stomach tube and the mice were killed at one week intervals. To obtain histological samples, the small intestine was excised and divided into the duodenum, jejunum and ileum, and then the worms were counted under a microscope.

2. Tissue fixation

The duodenum, jejunum and ileum from the entire small intestine were divided, washed with PBS and fixed with 10% formalin and Carnoy's solution at 4℃.

3. Immunohistochemistry

Immunohistochemistry was carried out using the anti-*c-kit* antibody (Santa Crutz, Santa Crutz, CA) for mast cells. Tissue sections of 5 μm were attached to poly-L-lysine (Sigma, St. Louis, MO) coated slides and deparafinized. After the removal of peroxidase, the tissues were incubated for 10 min with 3% H_2O_2 and they were subsequently incubated with Tris solution (pH 7.6) for 5 min. Tissues in citrate buffer (pH 6.0) were treated 3 times in a microwave oven for 5 min, cooled at room temperature and then washed with D.W. Non-specific antibody binding was reduced by incubating the tissues in 5% normal rabbit serum before the addition of the primary antibody. The tissues were then incubated with goat anti-*c-kit* mouse antibody at a 1:200 dilution for 1 hr, washed with Tris solution, incubated with rabbit anti-goat IgG (DAKO, DK, Glostrup, Denmark) and then incubated with streptavidin-peroxidase for 20 min. 3,3' diaminobenzidine (0.5 mg/ml) was used as the chromogen. We performed with the same procedures on the negative control except for the primary antibody incubation. Mayer's hematoxylin was used as the counterstain and the samples were mounted with canada balsam.

4. Periodic acid stain

The tissues were embedded in paraffin, cut with a microtome, stained with PAS and mounted with canada balsam for goblet cells.

5. Counting of mast cells and goblet cells

The number of mast cells and goblet cells was calculated as previously described[10]. 10 villi was counted in each region of intestine and all counts were expressed as the number of cells per villus-crypt unit (VCU).

6. Statistical analysis

Data are presented as mean ± SD. Statistical differences were analyzed by using Kruskal-Wallis test for the difference between mast cells and goblet cells, and Pearson's correlation coefficient test was used for the relationship of the worm recovery rate and mast cells or goblet cells. The SAS statistical software package was used for statistical analysis. The significant value is defined as $P<0.05$.

RESULTS

1. Worm recovery rate

Worm recoveries in the C3H/HeN mice were 65.7±5.6, 53.3±5.4, 6.7±0.6, 3.3±0.8, and 3.3±0.8% in week 1, 2, 3, 4, and 5 post-infection (P.I.), respectively and the rates quickly decreased in week 3 P.I. Worm recoveries in the BALB/c mice were 23.0±2.5, 10.0±1.0, and 6.7±0.6% in week 1, 2, and 3 P.I., respectively and the rates gradually lessened from week 1 P.I. to week 3 P.I. Worm recoveries in the C3H/HeN mice were significantly higher than in the BALB/c mice ($P<0.001$). No worms were recovered in the C3H/HeN and BALB/c mice after week 5 P.I (Table 1).

Table 1. Worm recovery rate (%) of *E. hortense* in mice

Week after infection	Mouse strain	
	C3H/HeN	BALB/c
1	65.7±5.6[a,**]	23.0±2.5
2	53.3±5.4[**]	10.0±1.0
3	6.7±0.6	6.7±0.6
4	3.3±0.8	N.D.[b]
5	3.3±0.8	N.D.
6	N.D.	N.D.
7	N.D.	N.D.
8	N.D.	N.D.

[a] mean ± SD, [b] N.D.: not detected, [**] $P<0.001$

2. Goblet cells in intestine of mice

1) The number of goblet cells in the intestine of C3H/HeN mice

The number of goblet cells in the duodenum of the control group was 170.0±28.6. In the *E. hortense*-infected group, the number increased rapidly in week 1 P.I. (250.3±40.2), it reached a peak in week 3 P.I. (267.3±27.0) and then declined until week 8 P.I. (Fig. 1A and Fig. 3A & B). In the jejunum of the control group and *E. hortense*-infected group, the number of goblet cells in week 1 P.I was 156±23.6 and 348.7±19.0, respectively. The number of goblet cells in the *E. hortense*-infected group reached a peak in week 3 P.I. (398.3±33.2) and it lessened gradually until week 8 P.I. (137.0±4.6). For the number of the goblet cells, there is significant difference between the control group and the *E. hortense*-infected group ($P<0.01$). The number of goblet cells in the ileum of the *E. hortense*-infected group (260.7±44.7) was higher in week 1 P.I. in comparison with the control group (171.7±18.0), and it reached a maximum level in week 4 P.I. (275.0±18.2) and decreased continuously until week 8 P.I. (145±26). The number in the control group and *E. hortense*-infected group showed a significant difference by statistical analysis ($P<0.01$).

Taken together, the number of goblet cells in the intestine of C3H/HeN mice increased from week 1 P.I. and reached a maximum level in week 3 P.I., and then it lessened. In week 7 P.I., the number in the *E. hortense*-infected group was the same as in the control group. The number in the jejunum of the *E. hortense*-infected group was higher than the number in the duodenum and ileum.

2) The number of goblet cells in the intestine of BALB/c mice

The number of goblet cells in the duodenum for the control group was 106.3±6.7 In the *E. hortense*-infected group, the number increased rapidly in week 1 p.i. (152.3±24.6), and it reached a peak in week 4 p.i. (196.3±18.4) and then it declined until week 8 p.i. (119.0±4.6) (Fig. 1B and Fig. 3C & D). The number between the control group and the *E. hortense*-infected group was shown to be significantly different by statistical analysis ($P<0.01$). There is no significant difference between the control group and the *E. hortense*-infected group in the number of goblet cells in the jejunum. The number of goblet cells in the ileum for the control group was 118.7±27.0. In the *E. hortense*-infected group, the number increased in week 2 p.i. (131.3±16.7), and it reached a peak in week 4 p.i. (163.3±8.4), and then it lessened until week 8 p.i. (145±26). The number in the control group and the *E. hortense*-infected group was shown to be a significant difference by statistical analysis ($P<0.01$). The number of goblet cells at the jejunum in the BALB/c mice was as high as in the C3H/HeN mice.

3. Mast cells in intestine of mice

1) The number of mast cells in the intestine of C3H/HeN mice

The number of mast cells in the duodenum of the control group was 20.0±2.0. In the *E. hortense*-infected group, the number increased rapidly in week 1 P.I. (146.0±15.1), and it reached a peak in week 3 P.I. (198.0±16.0), and then it declined until week 8 P.I. (Fig. 2A). The number of mast cells in the jejunum of the control group and the *E. hortense*-infected group was 20.2±4.1 and 120.0±3.3, respectively. The number in the *E. hortense*-infected group reached a maximum level in week 3 P.I. (132.0±6.4), and then it decreased gradually until week 8 P.I. The number of mast cells in the control group and the *E. hortense*-infected group showed a significant difference by statistical analysis ($P<0.01$). The number of mast cells in the ileum of the control group was 13.4±2.1. In the *E. hortense*-infected group, the number increased markedly and reached a maximum level in week 3 P.I. (121.0±5.3), and then it lessened until week 6 P.I.

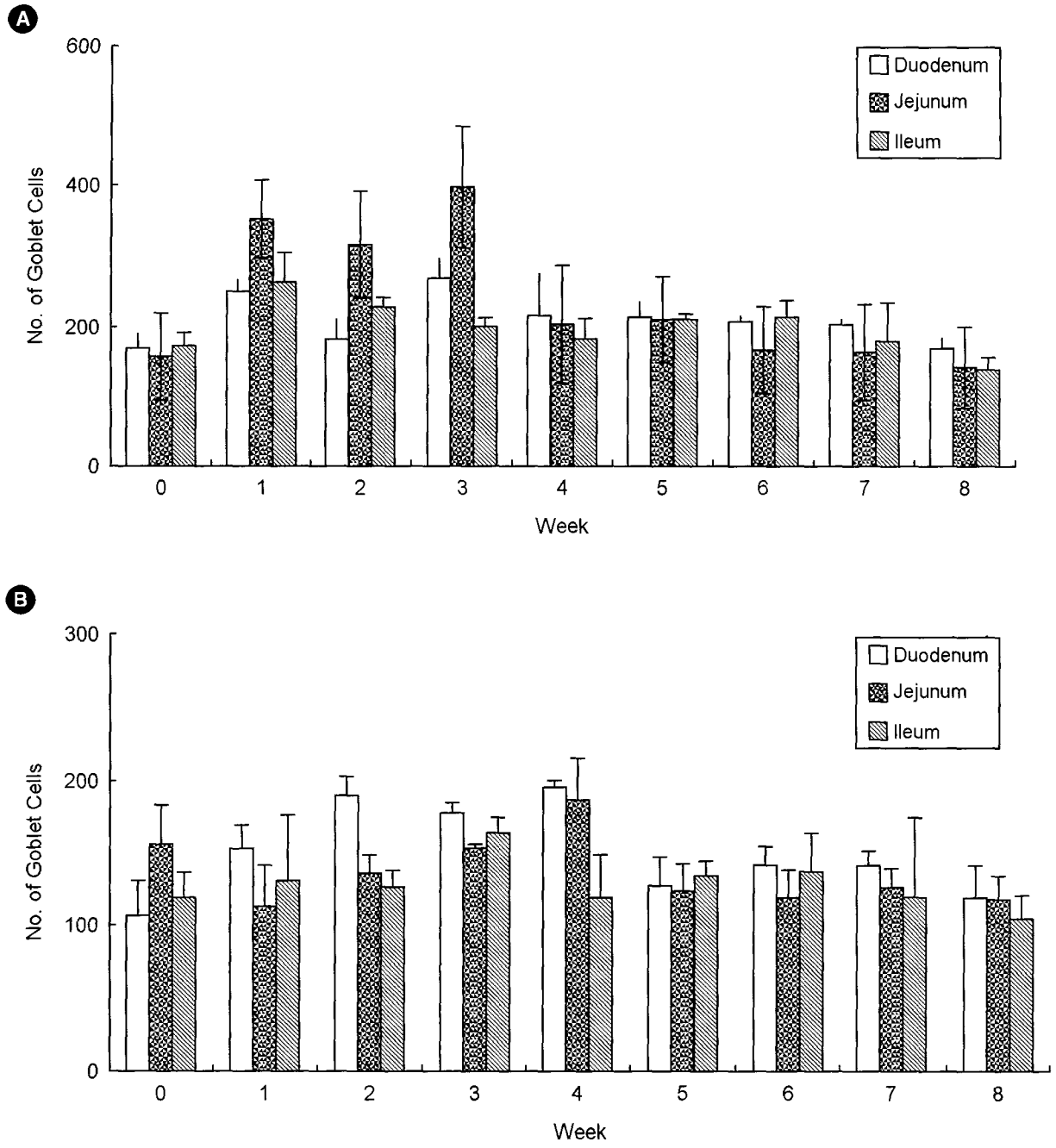

Fig. 1. Kinetics of the goblet cells at 3 regions of the intestine in C3H/HeN **(A)** and BALB/c mice **(B)**.

2) The number of mast cells in intestine of BALB/c mice

The number of mast cells in the duodenum of the control group was 19.3±3.1. In the *E. hortense*-infected group, the number increased rapidly from week 1 P.I. and reached a peak in week 3 P.I. (126.7±20.8), and then it decreased in week 6 P.I. as much as the control group (Fig. 2B). The number of mast cells between the control group and the *E. hortense*-infected group showed a significant difference by statistical analysis ($P<0.01$). The number of mast cells in the jejunum of the control group was 18.9±4.1. In the *E. hortense*-infected group, the number increased rapidly from week 1 P.I. and reached a peak in week 3 P.I. (116.0±7.5), and then it subsequently declined. The number of mast cells in the ileum of the control group was 15.0±2.6. In the *E. hortense*-infected group, the number increased rapidly from

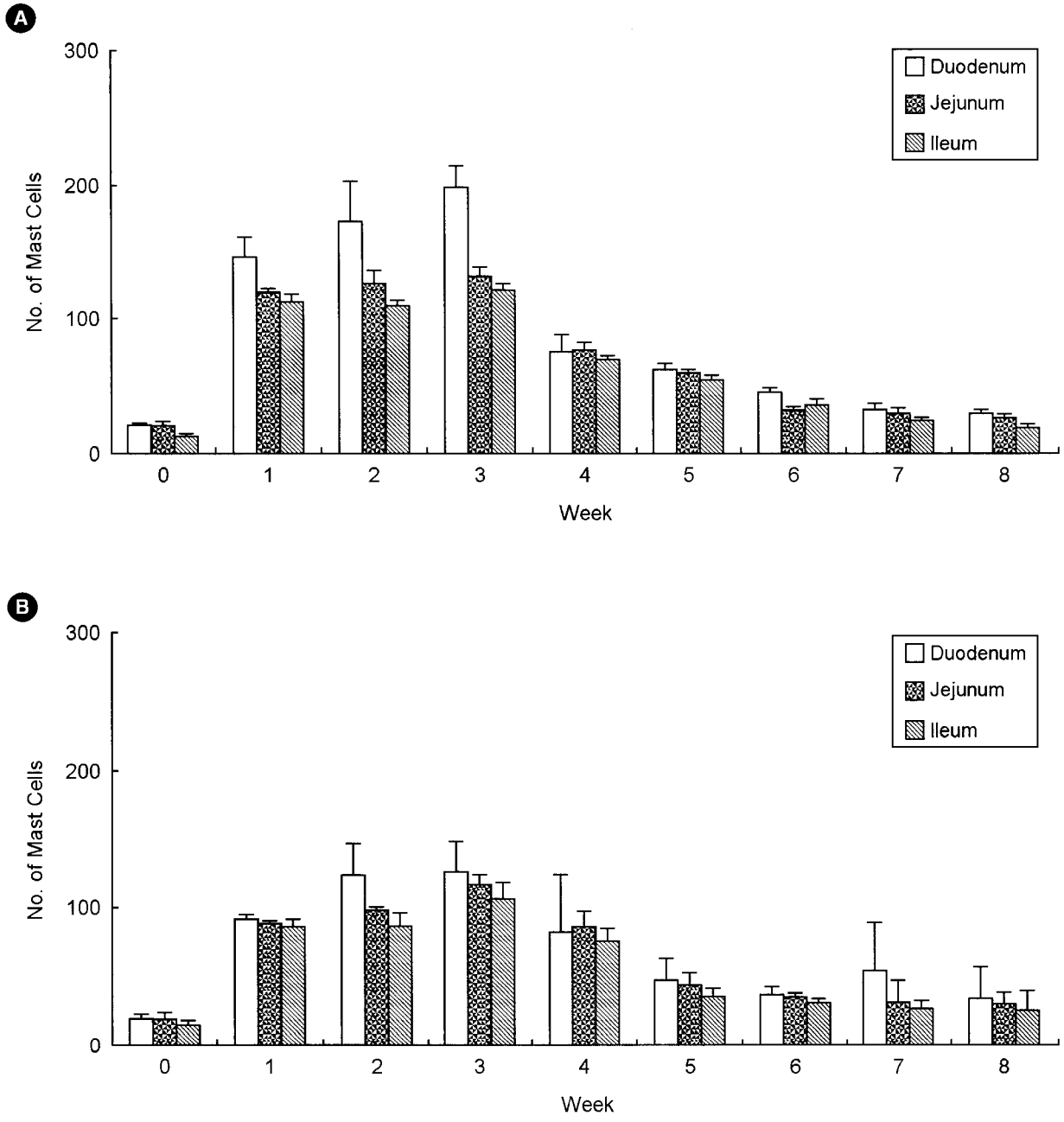

Fig. 2. Kinetics of the mast cells in intestine of C3H/HeN **(A)** and BALB/c mice **(B)**.

week 1 P.I. and reached a peak in week 3 P.I. (106.0±12.0), and then it decreased in week 5 P.I. as much as the control group. Taken together, the number of mast cells in C3H/HeN and BALB/c mice reached a peak in week 3 P.I. and recovered to a normal level in week 5 P.I. and 6 P.I., respectively. The number in the *E. hortense*-infected C3H/HeN mice (P=0.0015) was higher than in the *E. hortense* infected BALB/c mice (P=0.01) compared with the control group. In the number of mast cells, significant differences among regions of the intestine were found in the C3H/HeN mice (P<0.05), but not in BALB/c mice (P>0.05)

DISCUSSION

Worm expulsion by a host's immune response is associated with a variety of cells[14], and the sensitivity of the host

Fig. 3. Microphotographs of small intestinal villi showing goblet cells in C3H/HeN and BALB/c mice. Periodic Acid Schiff's (PAS) stain was performed to identify goblet cells in intestinal villi. **A** and **B**, C3H/HeN mice; **C** and **D**, BALB/c mice; **A** and **C**, *E. hortense*-non-infected experimental controls; **B** and **D**, 2 weeks after *E. hortense* infection. Original magnifications: ×100. 1, lumen; 2, villus, 3, lamina propria mucosa; 4, goblet cell; 5, lamina muscularis mucosa; 6, tela submucosa. Arrows indicate the goblet cells.

depends on the kind of parasite and species of host. Worm recoveries after week 1 in C3H/HeN and BALB/c mice infected with *E. hortense* was 65.7% and 23% respectively, indicating that worm expulsion in C3H/HeN mice was higher than in BALB/c mice. Chai et al. (1984)[2] reported that worm recovery in KK mice (18.9%) was higher than C3H/HeN mice (1.2%) after 1 week with being infected with *M. yokogavai*. Chai et al. (1998)[4] demonstrated that C57BL6 (H-2b) mice in worm expulsion of *N. seoulense* has a strong effect in comparison with BALB/c (H-2d) and C3H/HeN mice. In the present study, we investigated whether the change in the number of goblet cells and mast cells is associated with mouse strain and infection time. Our results demonstrated that the period (week 3 P.I.) in which worm recovery lessened rapidly was the same period in that the number of goblet cells and mast cells reached a peak. These results indicated that worm recovery significantly correlated with the growth of goblet cells and mast cells (P=0.0482) (Fig. 1 & 2). However, worm expulsion is not associated with goblet cells or mast cells in BALB/c mice. Recent studies have reported on the kinetics of mast cells in experimental animals infected with parasites and the results differed according to the kind of parasite and the host sensitivity[5]. Woodbury et al (1984)[19] reported that mast cells in rats infected with *T. spiralis* play an central role in worm expulsion. Worm recovery of *N. seoulense* is associated with increased mast cells[4]. However, there were no marked differences between worm recovery of *M. yokogavai* and mast cells[3]. These results were in line with our results that worm expulsion for *E. hortense* is not associated with an alternation of mast cells. The study by Chai et. al (1998)[4] demonstrated that worm repulsion of *N. seoulensis* in BALB/c mice was much higher than in C3H/HeN mice, but the number of mast cells in BALB/c mice was lower than in C3H/HeN mice. Goblet cells in BALB/c and C3H/HeN mice were all elevated. These results indicate that goblet cells and mast cells have no effect on worm recovery for *N. seoulensis* and any alternation of mast cells is

not associated with the mouse strain. Mast cells may function as a local immune response. The kinetics of mast cells and goblet cells is affected by the infecting parasite and the kind of host. Our results showing that the period (week 3 P.I.) of markedly decreased worm expulsion in C3H/HeN mice was correlated with the maximum level of goblet cells is in line with the results of Fujino et al. (1996) and Weinstein et al. (1991)[6,18].

This study performed immunohistochemistry using an anti-*c-kit* antibody for precise examination of mast cells. Hematoxylin-eosin (H-E) stain is difficult to use for the differential examination of mast cells and fibroblasts, histocytes, hairy cells and immatured granulocytes[17,21]. Both toluidine blue and alcian blue stain are useful methods for identifying metachromic factor, but the defect of these methods is that the examination must be performed as soon as possible because the metachromic granules disappear by the use of acidic dye for decalcification. Protooncogen *c-kit* (CD117) is stem cell factor and it increases the growth of mast cells. Recently, the *c-kit* was recommended as diagnostic factor for the examination of mast cells[9,20]. Immunohistochemistry using *c-kit* antibody is a useful method for the examination of mast cells[8].

In conclusion, we demonstrated that the rapid expulsion of *E. hortense* in C3H/HeN mice was associated with an increased number of mast cells and goblet cells, but not in BALB/c mice.

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