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Mucosal Mast Cell Responses in the Small Intestine of C3H/HeN and BALB/c Mice Infected with *Echinostoma hortense*

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In the intestinal mucosa, mast cells are thought to be responsible for the expulsion of parasites. We investigated the relationship of worm expulsion and mast cells in C3H/HeN and BALB/c mice infected with *Echinostoma hortense*. In addition, we examined whether the worm recovery rate was associated with the strain of mice, and whether a toluidine stain and immunohistochemistry using the *c-kit* antibody was effective in the detection of mast cells. In order to investigate the mucosal immune response of C3H/HeN and BALB/c mice, each mouse was infected orally with 30 *E. hortense* metacercariae. Then, the number of mucosal mast cells and worm recovery rates was observed in experimentally infected mouse strains between 1 week and 8 weeks post infection (PI). Mucosal mast cells were increased in 3 weeks P.I. in C3H/HeN and BALB/c mice. On the other hand, only mucosal goblet cells and worm recovery rates correlated in C3H/HeN mice (P=0.0482). Worm recoveries in C3H/HeN mice were 65.7±5.6, 53.3±5.4 and 6.7±0.6 in week 1, 2, and 3 P.I. and strongly decreased in week 3 P.I. Worm recoveries in 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. and gradually decreased from week 1 P.I. to week 3 P.I. Worm recoveries in C3H/HeN mice were significantly higher than in BALB/c mice (P<0.001). The number of mast cells in C3H/HeN and BALB/c mice using the anti-*c-kit* antibody reached to a peak in week 3 P.I. and recovered as normal level in week 5 P.I. and 6 P.I. The number in *E. hortense*-infected C3H/HeN mice (P=0.0015) was higher than in *E. hortense*-infected BALB/c mice (P=0.01) compared with the control group. There were significant differences in the number of mast cells among regions of the intestine in in C3H/HeN mice (P<0.05) but not in BALB/c mice (P>0.05). Immunohistochemistry using the anti-*c-kit* antibody was significant method as an examination of the number of mast cells (P=0.0002). In conclusion, the present study demonstrated that mast cells play an important role in worm recovery, and immunohistochemistry using the anti-*c-kit* antibody was superior to toluidine stain as an examination of mast cells.

Key Words: *Echinostoma hortense*, Mucosal mast cell, Worm recovery rate, C3H/HeN and BALB/c mice

INTRODUCTION

A host infected with a parasite does not show continuous lesions because the immune response of the host inhibits, degrades, or excretes the infected parasite. There are many reports about parasite expulsion, specifically with nematodes including *Trichinella spiralis*, *Nippostrongylus braziliensis*, *Strongyloides ratti* and trematoda including *Metagonimus yokogawai*, *Neodiplostomum seoulense*[3,16]. T cell-dependent and T cell-independent mechanisms are involved in parasite excretion, which happens within weeks after infection. The T cell-dependent mechanism increases cytokines, such as IL-4 and IL-5, and antibodies in response to parasites. In addition, IL-3, IL-4, IL-9, and IL10 increases the growth of mucosal mast cells and goblet cells. IgE-stimulated mast cells secrete histamine functioning increased the permeability of intestinal epithelial cells and increase parasite killing. Parasite expulsion is increased by mucosal mast cells, goblet cells, IgA secretion, and various cytokines. There is a variety of immune responses that are classified by the kind of parasite an thegenetic background of the host[13,16].

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Goblet cells play an central role in worm expulsion of *N. braziliensis*. However, mast cells play an essential role in *S. ratti* and *T. spiralis* infection[9,11,12,17]. Chai *et al.* (1993)[1,3] reported that the period in which worm expulsion in mice, infected with *M. yokogawai*, decreased markedly concorded with that of the maximum growth of mast cells. In rats infected with *N. seoulense* (*Fibricola seoulensis*), worm recovery rate was associated with the growth of mast cells[7].

In the current study, we investigated the relationship of worm expulsion and mast cells in C3H/HeN and BALB/c mice that were infected with *E. hortense*. In addition, we examined whether the worm recovery rate is associated with the strain of mice, and whether toluidine stain and immunohistochemistry, using the anti-*c-kit* antibody, is effective in the detection of mast cells.

MATERIALS AND METHODS

1. Metacercariae infection and worm recovery rate

Both 6 week old BALB/c and C3H/HeN mice were obtained from the Korean Experimental Animal Center. Experimental groups were divided into the control group and the *E. hortense*-infected group. Five mice were included in each group. Metacercariae of *E. hortense* were collected by artificial digestion of *Misgurnus anguillicaudatus* that caught a Munmakcheon. Each mouse was fed with thirty metacercariae via the stomach tube, and infected mice were killed at intervals of one week. For immunohistochemical samples, the small intestine was divided into the duodenum, jejunum, and ileum and was excised. The worms were counted using a microscope.

2. Examination of mast cells

Both the toluidine stain and immunohistochemistry using the anti-*c-kit* (CD117) antibody were performed to observe the mast cells. The duodenum, jejunum, and ileum from the small intestine were divided, washed with PBS, and fixed with Carnoy's solution at 4℃. Fixed tissues were deparafinized, made as a tissue section, and mounted with Canada balsam. Examination under the microscope was carried out within 2 h after the stain. Immunohistochemistry was carried out using monoclonal antibodies to *c-kit* (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 were deparafinized. For the removal of peroxidase in tissues, tissues were incubated for 10 min with 3% H_2O_2 and subsequently were incubated with Tris solution (pH 7.6) for 5 min. Tissues in the citrate buffer (pH 6.0) were treated 3 times in a microwave oven for 5 min, cooled at room temperature, and washed with distilled water. Non-specific antibody binding was reduced by incubating the tissues in 5% normal rabbit serum before the addition of the primary antibodies. 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 incubated with streptavidin-peroxidase for 20 min. 3,3' diaminobenzidine (0.5 mg/ml) was used as the chromogen. The negative control was performed using the same procedures with the exception of the primary antibody incubation. Mayer's hematoxylin was used as the counterstain and was mounted.

3. Counting of mast cells

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

4. Statistical analysis

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

RESULTS

1. Worm recovery rate

Worm recoveries in 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 (PI), respectively. There was a strong decrease in week 3 P.I. Worm recoveries in 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 there was a graduall decrease from week 1 P.I. to week 3 P.I. Worm recoveries in C3H/HeN mice were significantly higher than in BALB/c mice ($P<0.001$). In week 5 P.I., no worm was recovered in C3H/HeN or BALB/c mice (Fig. 1).

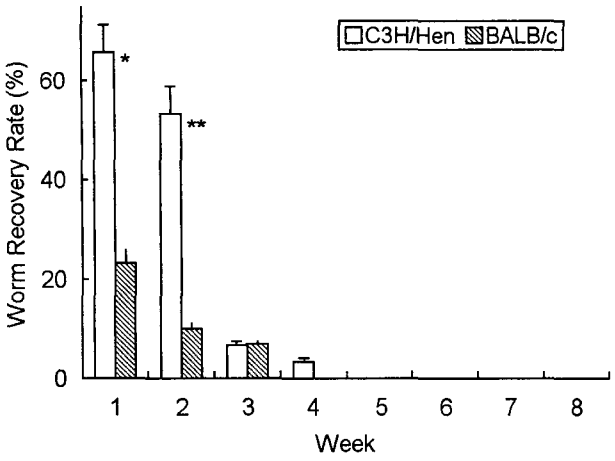

Fig. 1. Worm recovery rates (%) of *E. hortense* in mice.
Data were expressed as mean values±SD for mice. *P<0.001 **P<0.001

2. Examination of mast cells in mice by immunostain using the anti-*c-kit* antibody

1) The number of mast cells in 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), reached a peak in week 3 P.I. (198.0±16.0), and then declined until week 8 P.I. (Fig. 2).

The number of mast cells in the jejunum in 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 to a maximum level in week 3 P.I. (132.0±6.4) and decreased gradually until week 8 P.I. (Table 1).

The number of mast cells in the control group and the *E. hortense*-infected group showed a significant difference through statistical analysis (P<0.01).

The number of mast cells in the ileum in the control group was 13.4±2.1. In the *E. hortense*-infected group, the number increased markedly, reached the maximum level in week 3 P.I. (121.0±5.3), and then lessened until week 6 P.I.

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

The number of mast cells in the duodenum in the control group was 19.3±3.1. In the *E. hortense*-infected group, the number increased rapidly from week 1 P.I. reached the peak in week 3 P.I (126.7±20.8), and decreased in week 6 P.I. as much as the control group (Fig. 2). The number of mast cells in the control group and the *E. hortense*-infected group showed a significant difference through statistical analysis (P<0.01).

The number of mast cells in the jejunum in the control group was 18.9±4.1. In the *E. hort*ense-infected group, the number increased rapidly from week 1 P.I., reached the peak in week 3 P.I. (116.0±7.5), and then decreased.

The number of mast cells in the ileum in the control group was 15.0±2.6. In the *E. hortense*-infected group, the number increased rapidly from week 1 P.I., reached the peak in week 3 P.I. (106.0±12.0), and decreased in week 5 P.I. as much as the control group (Table 1). Taken together, the number of mast cells in C3H/HeN and BALB/c mice reached the peak in week 3 P.I. and recovered to the normal level in week 5 P.I.and 6 P.I. The number in the *E. hortense*-infected C3H/HeN mice (P=0.0015) was higher than the number in the *E. hortense*-infected BALB/c mice (P=0.01), but both were higher compared with the control group. In the number of mast cells, significant differences among regions of the intestine were present in C3H/HeN mice (P<0.05) but were not in BALB/c mice (P>0.05).

3. Examination of mast cells in mice by toluidine stain

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

The number of mast cells in the duodenum in the control group was 3.3±1.5. In the *E. hortense*-infected group, the number increased in week 1 P.I. (11.0±2.0), reached the peak in week 3 P.I. (20.3±3.5), and then, declined until week 8 P.I. (9.7±3.8) (Fig. 2).

The number of mast cells in the jejunum in the control group and the *E. hortense*-infected group was 2.0±1.0 and 7.7±4.0, respectively. The number in the *E. hortense*-infected group reached the maximum level in week 3 P.I. (13.7±4.6), and decreased gradually until week 8 P.I. (5.3±1.5).

The number of mast cells in the ileum in the control group was 2.0±1.0. In the *E. hortense*-infected group, the number increased markedly, reached the maximum level in week 3 P.I. (8.7±4.0), and then lessened until week 8 P.I. (6.0±2.6).

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

The number of mast cells in the duodenum in the control

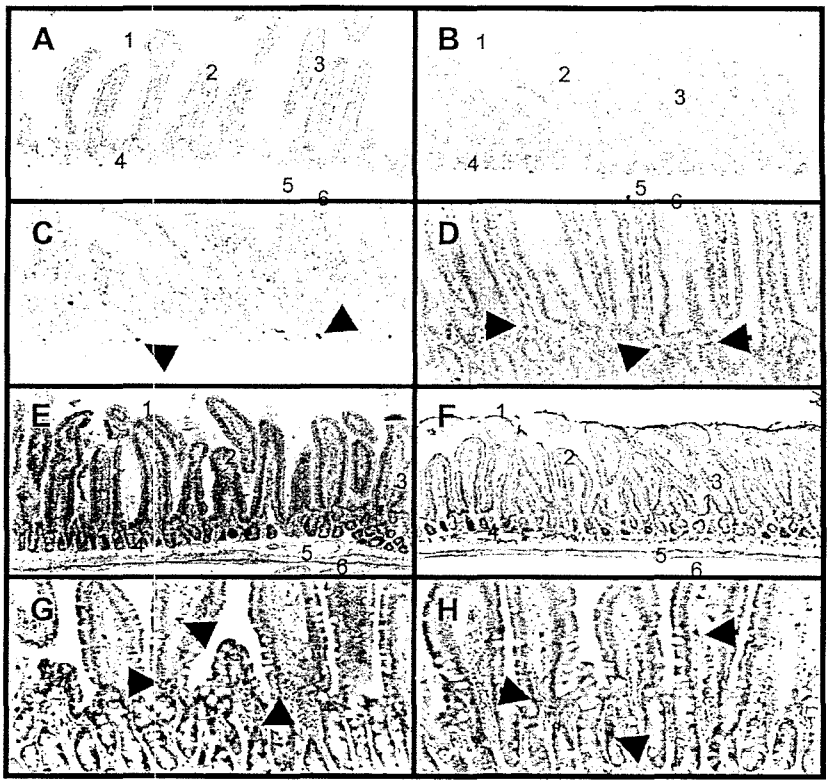

Fig. 2. Microphotographs of small intestinal villi showing mast cells in C3H/HeN and BALB/c mice. **A, C, E,** and **G:** C3H/HeN mice; **B, D, E,** and **F:** BALB/c mice: **A** and **B:** toluidine blue control; **C** and **D:** toluidine blue 2 weeks; **E** and **F:** experimental control; **G** and **H:** immunohistochemical staining for *c-kit* at 2 week post infection. Original magnifications: **A, B, E,** and **F:** × 100. **C, D, G,** and **H:** × 200. 1, lumen; 2, villus, 3, lamina propria mucosa 4, goblet cell; 5, lamina muscularis mucosa 6, tela submucosa. Arrows indicate the intraepithelial lymphocytes.

Table 1. Kinetics of the average number of total mast cells with c-kit (CD117) immunohistochemical stain in the intestine of C3H/HeN and BALB/c mice

Weeks after infection	C3H/HeN*			BALB/c**		
	duodenum	jejunum	ileum	duodenum	jejunum	ileum
control	20±2.0*	20.2±4.1	13.4±2.1	19.3±3.1	18.9±4.1	15.0±2.6
1	146±15.1	120±3.3	113±5.0	91.3±3.1	88.0±2.3	85.0±6.1
2	173±30	125±11.1	109±5.3	123.3±23.1	99.0±1.5	86.0±9.3
3	198±16	132±6.4	121±5.3	126.7±20.8	116±7.5	106±12
4	75.3±13.4	77±5.2	69±3.9	80.7±42.4	85.0±12.3	76.0±8.4
5	62.3±4.7	60±2.4	55±3.3	46.7±15.3	44.0±7.7	35.0±6.0
6	45.3±3.1	33.5±1.5	36±4.3	36.7±5.8	35.4±2.9	30.0±3.5
7	33±3.5	30±4.1	25±2.1	53.3±35.1	31.0±16	26.8±4.7
8	29.7±3.5	28±1.9	19±3.5	33.3±23.1	29.0±8.9	25.0±13.5

*P<0.015, **P<0.05

group was 3.0±1.0 In the *E. hortense*-infected group, the number increased rapidly from week 1 P.I. and reached to a peak in week 6 P.I. (9.3±3.2) (Fig. 2). The number of mast cells in the *E. hortense*-infected group between 6 P.I. and 8 P.I. showed a significant difference by statistical analysis (P<0.01).

The number of mast cells in the jejunum in the control group was 3.3±2.5. The number of mast cells in the control

group and the *E. hortense*-infected group was significantly different according to statistical analysis ($P<0.01$).

The number of mast cells in the ileum in the control group was 2.3±2.5. The number of mast cells in the *E. hortense*-infected group showed a significant difference among infection durations through statistical analysis ($P<0.01$).

In summary, the number of mast cells in C3H/HeN mice reached a maximum level in week 3 P.I. and gradually lessened. The number in *E. hortense*-infected C3H/HeN mice were higher than the number in *E. hortense*-infected BALB/c mice, but both were higher compared with the control group. In the number of mast cells, significant differences among regions of the intestine were in C3H/HeN mice ($P=0.00285$) but were not in BALB/c mice ($P=0.19$).

4. Comparison immunohistochemistry and toluidine stain in the examination of mast cells in mice

The examination of mast cells using immunohistochemistry detected higher than using the toluidine stain. The number of mast cells in the duodenum of the *E. hortense*-infected group using immunohistochemistry (198.0±16.0) was higher than the number obtained using the toluidine stain (20.3±3.5). Immunohistochemistry using the anti-*c-kit* antibody is significant difference ($P=0.0002$) in the number of mast cells but not toluidine stain.

DISCUSSION

Various immune cells in the host are included in worm expulsion[14], and immune sensitivity of the host is associated with the kind of parasite and species of host. Worm recoveries in C3H/HeN and BALB/c mice after being infected for 1 week with *E. hortense* were 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 of infection with *M. yokogawai*. Chai *et al.* (1998)[4] demonstrated that C57BL6 (H-2b) mice, in worm expulsion of *N. seoulense*, has a prominent effect in comparision with BALB/c (H-2d) and C3H/HeN mice. In the present study, we investigated whether the alternation of 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 lessen rapidly is the same period that the number of mast cells reaches its peak, indicating that worm recovery significantly correlates with the growth of mast cells ($P=0.0482$). However, worm expulsion is not associated with mast cells in BALB/c mice. Recent studies reported kinetics of mast cells in experimentally infected animals with parasites and reported different results depending on the kind of parasite and host sensitivity[15]. Woodbury *et al.* (1984)[19] reported that mast cells in rats that were infected with *T. spiralis* play an essential role in worm expulsion. Worm recovery of *N. seoulense* is associated with an increase in mast cells[4]. However, there were no marked differences between worm recovery of *M. yokogawai* and mast cells (Chai, 1993)[3]. These results were in line with our results that worm expulsion of *E. hortense* is not associated with the alternation of mast cells. Goblet cells play an essential role in normal and athymic BALB/c mice and C3H/HeN mice infected with *E. trivolvis*, but mast cells do not[5,6]. Weistein *et al.* (1991)[18] also reported that goblet cells in BALB/c and C3H/HeN mice reached the peak on day 13 P.I. and were related with worm expulsion. 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 both BALB/c and C3H/HeN mice increased. These results indicate that goblet cells and mast cells have no effect on worm recovery of *N. seoulensis*, and the alternation of mast cells is not associated with mouse strain. Mast cells may function as a local immune response. Kinetics of mast cells and goblet cells are affected by the infected parasite and the kind of host. This study used both toluidine stain and immunohistochemistry with the anti-*c-kit* antibody for examination of mast cells. Hemoatoxylin-eosin stain is difficult to use for differential examination of mast cells and fibroblasts, histocytes, hairy cells, and immature granulocytes [17,21]. Both toluidine blue and alcian blue stains are useful methods for identify a metachromic factor, but the downside of these methods is the examination must be performed immediately because the metachromic granules disappear due to decalcification by acidic dye.

Protooncogen *c-kit* (CD117) is a stem cell factor and increases the growth of mast cells. Recently, *c-kit* was recommended as diagnosis by examination of mast cells [9,20]. Immunohistochemistry using the *c-kit* antibody is a useful

technique for the examination of mast cells[8].

In conclusion, the present study demonstrated that mast cells play an important role in worm recovery, and immunohistochemistry using anti-*c-kit* antibody is superior to the toluidine stain in examination of mast cells. This study was supported by the grant of Maeji Institute of Academic Research in fiscal year of 2002.

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