J. Exp. Biomed. Sci. 11 (2005) 259-266

Reaction of Mast Cells and Goblet Cells in the Small Intestine of C57BL/6 and C3H/HeN Mice Infected with *Echinostoma hortense*

Kyeong-Yeol Park[1], Kyu-Jae Lee[2], In-Sik Kim[3], Eun-Ju Yang[1], Su-jung Lim[1], Byung-Hyuk Lim[1] and Yong-Suk Ryang[1†]

[1]Department of Biomedical Laboratory Science and Institute of Health Science, College of Health Science, Yonsei University, Kangwon-Do 220-710, Korea. [2]Department of Parasitology, Wonju college of Medicine, Yonsei University, Kangwon-Do 220-701, Korea. [3]Department of Clinical Laboratory Science, School of Medicine, Eulji University, Daejeun 301-714, Korea

Mast cells and goblet cells have been known to protect the host against parasites. In this study, we examined the response of the mast cells and goblet cells over a period of 6 weeks in the duodenum, jejunum and ileum of C3H/HeN and C57BL/6 mice infected with *Echinostoma hortense* (*E. hortense*). In addition, we investigated whether the worm recovery rate of uninfected mice (the control group) or *E. hortense*-infected mice (the experimental group) was associated with the number of mast cells and goblet cells. The worm recovery rate was higher in the C3H/HeN mice than in the C57BL/6 mice. The number of goblet cells significantly increased in the experimental group of the C3H/HeN and C57BL/6 mice compared with the control group of both strains ($P<0.005$). Worm recovery peaked 3 weeks after the infection of the C57BL/6 mice and at 2 weeks after the infection of the C3H/HeN mice, and it was higher in the duodenum than in the jejunum and ileum. However, the infected site in the intestine had no relation with worm expulsion. In the C3H/HeN and C57BL/6 mice, the number of goblet cells in the experimental group was significantly higher than that in the control group ($P<0.005$). The number reached a peak 2 weeks after the infection and it even increased in duodenum, jejunum and ileum. The increased number of goblet cells was retained 6 weeks after infection. The number of goblet cells was higher in the C3H/HeN mice than in the C57BL/6 mice ($P<0.01$). These results indicate that goblet cells are related with the worm expulsion. Furthermore, immunohistostaining of the antral intestinal walls for lectin showed the significant increase of the number of goblet cells in the experimental group ($P<0.001$). The high infection rate in the duodenum was found during the early infection. An increased infection rate in the jejunum and ileum was found 3 weeks after infection and the infection rate was higher in the C3H/HeN mice than in the C57BL/6 mice. Taken together, the present study indicates that goblet cells, rather than mast cells, may play critical roles in parasite expulsion.

Key Words: *Echinostoma hortense*, Worm recovery rate, Mast cells, Goblet cells, C57BL/6 mice, C3H/HeN mice

INTRODUCTION

Mast cells in intestine mucosa play important roles in the immune response of the host, including the degranulation that is caused by parasites' antigenicity. A nematoda infection increases the number of mast cells and the histamine secretion in the early period of infection. At the later infection period, the number of mast cells recovers to a basal level, and this indicates what function the mast cells serve in early stage of parasite infection (Barrett et al., 1988). Mast cells are associated with allergic hypersensitivity and ion transport in the intestine (Harari et al., 1987; Perdue et al., 1990). Goblet cells exist in the columnar or pseudostratified columnar cells of intestine mucosa and they increase the production of mucin and glycoprotein, and these substances function to protect the host against invading microorganisms (Meslin et al., 1999). Many studies have reported

*Received: August 5, 2005
Accepted after revision: Ausgust 10, 2005
†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

that the hypertrophy of goblet cells and the increased mucin secretion is associated with parasite expulsion, and specifically for the nematode family including *Schistosoma mansoni, Trichinella spiralis, Nippostrongylus brasiliensis, Strongyloides ratti, Trichuris muris, Echinostoma trivolvis* and *trematoda* (Miller et al., 1979; Carroll et al., 1984; Miller, 1987; Grencis et al., 1991; Garside et al., 1992; Fujino et al., 1993; Khan et al., 1995; Ishikawa et al., 1997; Else et al., 1998; Fallon et al., 2000; Khan et al., 2001; Khan et al., 2003). It has been reported that the alteration of the mucin terminal sugar during worm expulsion is a more essential factor than the elevated number of goblet cells, as was determined by performing lectin histochemistry (Ishikawa et al., 1993; Ishikawa et al., 1994; Onah et al., 2000). Goblet cells in mice infected with *N. brasiliensis* strongly bind to lectin compared with goblet cells in normal mice, indicating that the goblet cells in infected mice have altered their mucins' terminal sugar.

In the current study, we examined the worm recovery rate and the alteration of goblet cell numbers and mucin secretion in C57BL/6 and C3H/HeN mice that were infected with *Echinostoma hortense*.

MATERIALS AND METHODS

1. Experimental animals and metacercaria infection

C57BL6 and C3H/HeN mice that were 5 weeks old were obtained from the Korean Experimental Animal Center. The experimental groups were divided into the control group and the *E. hortense*-infected group. *E. hortense* metacercariae were collected by an artificial digestion of *Misgurnus anguillicaudatus*. Thirty metacercariae were fed to each mouse via a stomach tube.

2. Worm recovery rate

The mice that were infected with metacercariae were killed at one week intervals for 6 week. For recovering the worms, the small intestine was excised, placed in 0.85% saline and incubated on ice for 2 hrs, and then the worms were collected.

3. Mast cell response in the intestine mucosa

Immunohistochemistry was carried out using the anti-c-kit (anti-CD117) antibody (Santa Crutz, Santa Crutz, Ca) for the mast cells. Tissue sections 5 μm thick were attached to poly-L-lysine (Sigma, St. Louis, MO) coated slides and then the sections were deparafinized. The tissues were incubated for 10 min with 3% H_2O_2 for the removal of peroxidase 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. The 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 3 times for 5 min with Tris solution, incubated with biotin-conjugated rabbit anti-goat IgG (DAKO, 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 the same procedures on the negative control except for the primary antibody incubation. Mayer's hematoxylin was used as the counterstain after developing and the samples were mounted with canada balsam.

4. Goblet cell response in the intestine mucosa

The duodenum, jejunum and ileum from the entire small intestine were divided into 2 cm sections and fixed with Carnoy's solution. The tissues were embedded in paraffin, cut with a microtome and stained with PAS. Hematoxylin was used as the counterstain. The number of goblet cells was analyzed according to a previously described method (Miller et al., 1987). 10 villi were counted in each region of the intestine and all counts were presented as the number of cells per villus-crypt unit (VCU).

5. Mucin response of goblet cells

Lectin immunohistochemistry was carried out with using helix pomatia agglutinin (HPA; Sigma, St. Louis, MO), as described by manufacturer's instruction, for examining the goblet cell activation. HPA binds to the modified N-Acetyl-D-galactosamine on GC mucin. The tissue sections were fixed with Carnoy's solution, embedded with paraffin and then sectioned to 4 μm thickness. The tissues were deparafinized, hydrolysed with ethanol and incubated for 20 min with 0.3% H_2O_2 in methanol for the removal of the peroxidase activity. After washing the sections 3 times with 0.01 M PBS (pH 7.4), the non-specific antibody binding was diminished by incubating the tissues in 1% bovine serum albu-

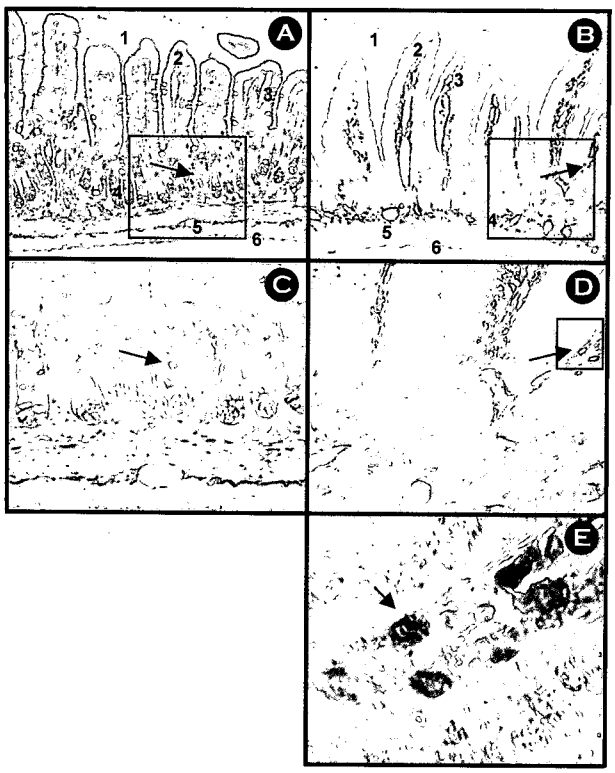

Fig. 1. Microphotographs of intestinal villi showing the c-kit-stained mast cells at 3 week after *E. hortense* infection in the C57BL/6 and C3H/HeN mice. **A** and **C**: the C3H/HeN mice; **B, D** and **E**: the C57BL/6 mice; Original magnifications: **A** and **B** = ×100; **C** and **D** = ×400; **E** = ×1000. 1, lumen; 2, villus; 3, lamina propria mucosa; 4, goblet cell; 5, lamina muscularis mucosa; 6, tela submucosa. Arrows indicate the mast cells.

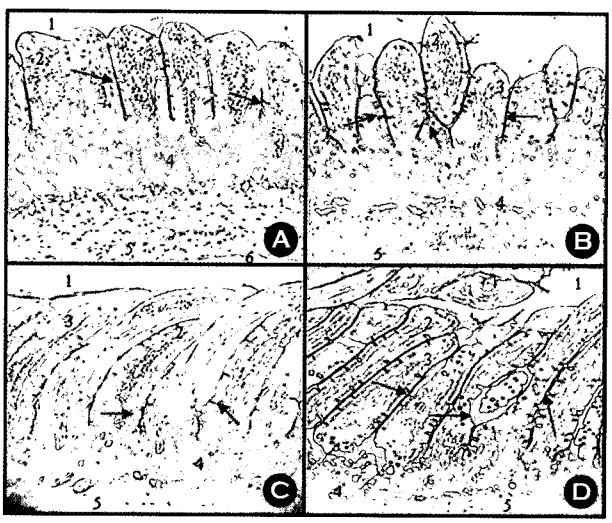

Fig. 2. Microphotographs of intestinal villi showing the goblet cells in the C57BL/6 and C3H/HeN mice. Periodic acid schiff's (PAS) staining was performed to identify the goblet cells in the small intestinal villi. **A** and **B**: C57BL/6 mice; **C** and **D**: C3H/HeN mice; **A C** and **E**: hortense-non-infected experimental controls; **B** and **D**: 3 weeks after *E. hortense* infection. Original magnifications: **A, B, C** and **D** = ×100; 1, lumen; 2, villus; 3, lamina propria mucosa; 4, goblet cell; 5, lamina muscularis mucosa; 6, tela submucosa. Arrows indicate the goblet cells.

min. The tissue sections were incubated at a biotinylated HPA concentration of 25 μg/ml in a moist chamber for 2 hours, then they were washed with PBS solution and incubated with biotin-conjugated rabbit anti-goat IgG (DAKO, Glostrup, Denmark). After incubated with streptavidin-HRP conjugate (Zymed, San Francisco, U.S.A) at a 1:200 dilution for 2 h, the tissues were developed. Mayer's hematoxylin was used as the counterstain. All counts were expressed as the number of HPA-positive cells per 10 VCU.

6. Statistical analysis

The data are presented as means ± SD. Statistical differences were analyzed by using the Student-t test for determining the difference between the control and experimental

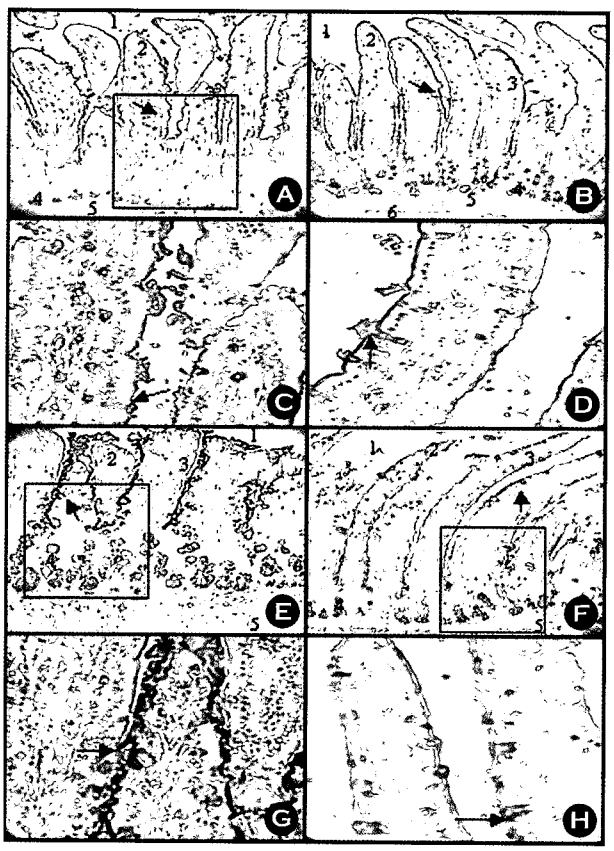

Fig. 3. Microphotographs of the intestinal villi showing the HPA-positive cells in the C57BL/6 and C3H/HeN mice. Lectin histochemistry was performed to identify the HPA-positive cells in the small intestinal villi. **A, C, E** and **G**: C3H/HeN mice; **B, D, F** and **H**: C57BL/6; **A, B, C** and **D**: experimental control; **E, F, G** and **H**: 3 weeks after *E. hortense* infection. Original magnifications: **A, B, E** and **F** = ×100. **C, D, G** and **H** = ×400. 1, lumen; 2, villus; 3, lamina propria mucosa; 4, goblet cell; 5, lamina muscularis mucosa; 6, tela submucosa. Arrows indicate the HPA positive cells.

Table 1. Comparison of the worm recovery rates from the C57BL/6 and C3H/HeN mice infected with *E. hortense*.

Mouse strain	Worm recovery rates at post-infection (%)					
	1 week	2 week	3 week	4 week	5 week	6 week
C57BL/6	59.0±6.7*	52.3±5.7	4.3±0.2	-	-	-
C3H/HeN	65.7±4.0	62.3±4.0	7.7±0.2	4.3±0.2	2.3±1.7	-

* mean ± SD

Table 2. Kinetics of the average number of total mast cells with *c-Kit* (CD117) immunohistochemical staining in the small intestine of the C57BL/6 and C3H/HeN mice infected with *E. hortense*

Weeks after infection	C57BL/6			C3H/HeN		
	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
0	20.3±0.5	18.9±5.0	10.1±3.9	19.0±0.8	18.2±2.0	14.3±4.3
1	91.7±6.2***	13.0±4.0	9.0±2.0	97.0±18.7	18.7±8.0	15.0±2.0
2	100.3±8.6***	32.7±7.0	16.0±3.6	100.0±18.5***	21.0±5.6	19.7±9.0
3	123.7±6.3	67.3±7.5	40.3±10.5	98.3±14.3	78.7±18.5	40.3±10.5
4	79.7±7.8	27.3±7.5	51.7±9.5	58.7±9.8	75.3±11.6	53.7±19.0
5	43.7±6.3	35.3±10.5	17.7±9.5	45.0±6.4	24.3±10.0	17.3±9.1
6	35.7±3.7	26.7±11.6	11.3±3.2	39.7±9.6	35.3±5.5	12.7±2.1

* mean ± SD

groups. The SPSS statistical software package (Version 10.0, Chicago, IL) was used for statistical analysis. *P values* < 0.05 were deemed statistically significant.

RESULTS

1. Worm recovery rate

Worm recovery was performed by collecting the worms after killing the mice infected with metacercariae at one week intervals for 6 week. The number of worms recovered in the C57BL/6 mice were 17.7±2.0, 15.7±1.7 and 1.3± 0.5 at 1, 2 and 3 weeks post-infection (P.I.), respectively. No worms were recovered after week 4 P.I. The number of worm recovered in the C3H/HeN mice was 19.7±1.2, 18.7 ±1.2, 2.3±0.5, 1.3±0.5 and 0.7±0.5 at 1, 2, 3, 4 and 5 weeks P.I., respectively. The rates gradually lessened from week 1 P.I. to week 3 P.I. The worm recovery rates in the C3H/HeN mice were higher than in the C57BL/6 mice (Table 1).

2. Mast cell response in the intestine mucosa

Mast cells were detected by performing immunohistochemistry with using anti-c-kit antibody in the C57BL/6 and C3H/HeN mice infected with *E. hortense* metacercariae. The mast cells were seen as dense brown spots on the electron microscopy (Fig. 1). The number of mast cells in the duodenum of the control group of the C57BL/6 mice was 20.3±0.5. In the *E. hortense*-infected group, the number of mast cells increased in week 1 P.I., and it reached a peak in week 3 P.I. (123.7±6.3) and then it declined until week 6 P.I. (35.7±3.7). In the C3H/HeN mice, the number of mast cells in the duodenum of the control group was 19±0.8. The number became elevated at week 1 P.I. and it reached a maximum level at week 2 P.I. (100.0±18.5), and then it declined until week 6 P.I. (39.7±9.6). Taken together, the mast cells in the intestine of the C3H/HeN mice were more increased than in the intestine of the C57BL/6 mice. In the *E. hortense*-infected group, the number of mast cells in the duodenum was higher than the number of mast cells in the jejunum and ileum (Table 2).

3. Goblet cell response in the intestine mucosa

The number of goblet cells was 104.3±13.2 in the duodenum of the control group of the C57BL/6 mice. The number increased rapidly in week 1, and it reached a peak in week 3 P.I. (169.3±8.2) and then it declined until week 6 P.I. The number of goblet cells was 109.7±11.8 in the duodenum of the control group of the C3H/HeN mice. In the *E. hortense*-infected group, the number of goblet cells continued to increase until week 3 P.I.; it reached a peak in

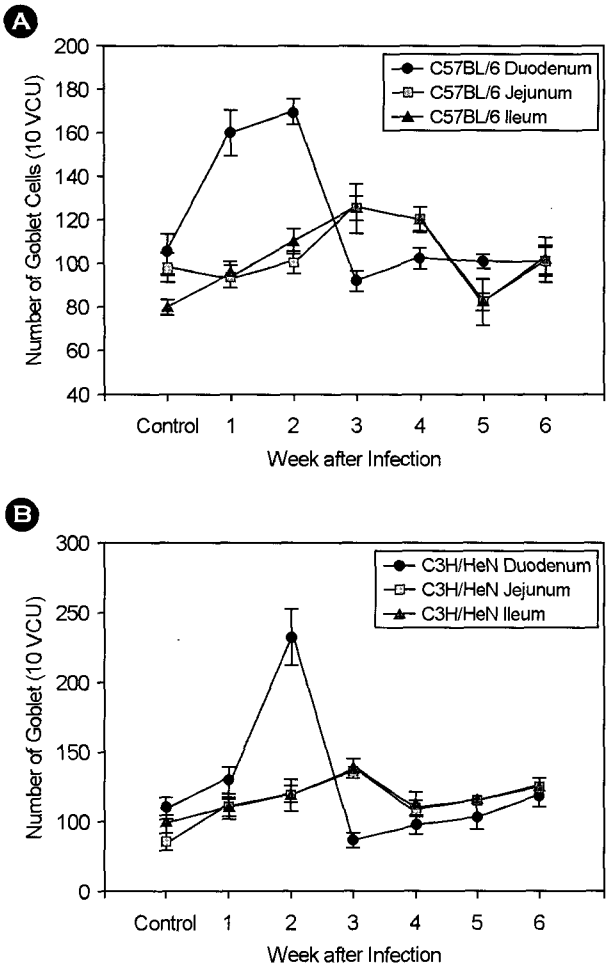

Fig. 4. Comparison of the number of goblet cells per 10 villus-crypt units (VCU) in the small intestine of the C57BL/6 and C3H/HeN mice infected with *E. hortense*.

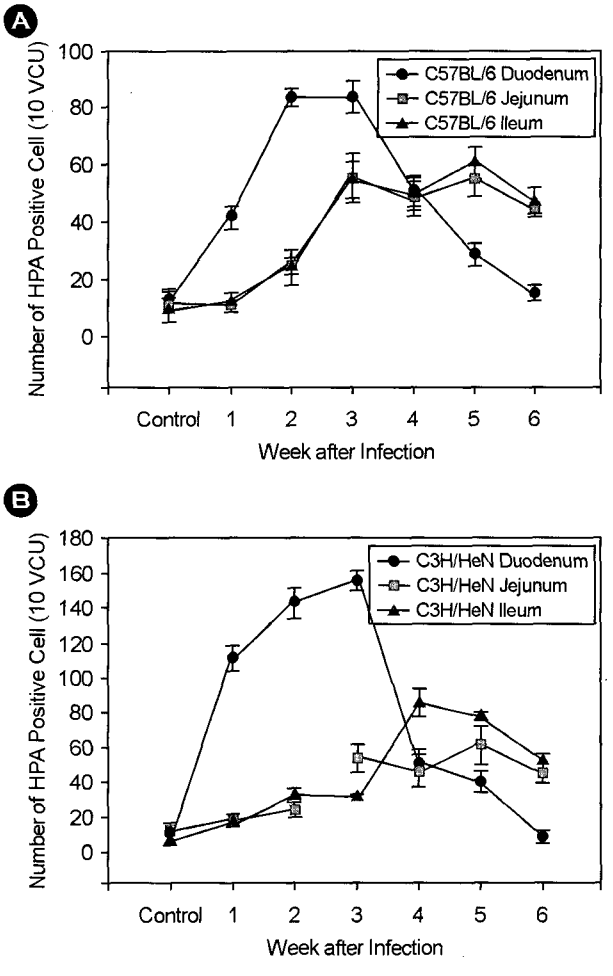

Fig. 5. Comparison of the number of HPA-positive cells per 10 villus-crypt unit (VCU) in the small intestine of the C57BL/6 and C3H/HeN mice infected with *E. hortense*.

week 3 P.I. (233.3±28.7) and then it declined. However, the number of goblet cells (120.0±12.2) in week 6 P.I was increased rather more than in week 5 P.I. (Fig. 2 and 4). (ED note: check this.) The goblet cells in the C3H/HeN mice were more increased than in the C57BL/6 mice. The number of goblet cells in the control group and in the *E. hortense*-infected group was shown to be significantly different by statistical analysis ($P<0.01$), however, this increased number was not related with the region of the intestine.

4. Mucin response of the goblet cells using lectin histochemistry

In the duodenum of the control group of the C57BL/6 mice, the number of HPA-positive cells was 11.7±2.5.; the number of HPA-positive cells in the *E. hortense*-infected group increased from week 1 and it reached a peak in week 2 and 3 P.I. (83.7±4.5 and 84.7±8.2, respectively), and then it declined until week 6 P.I. (15.0±3.7). The numbers in the jejunum and ileum of the control group of C57BL/6 mice were 10.3±3.9 and 9.7±8.4, respectively, and these numbers increased in week 5 P.I. (55.0±11.0, 60.7±9.0) (Fig. 3 and 5). The number of HPA-positive cells in the duodenum of the control group of the C3H/HeN mice was 8.7±1.2. In the *E. hortense*-infected group, the number reached a maximum level in week 3 P.I. (155.7±8.7) and then it fell until week 6 P.I. (12.0±2.4). The numbers of HPA-positive cells in the jejunum and ileum of the C57BL/6 mice were elevated until week 5 P.I. (Fig. 3 and 5). Taken together, the HPA-positive cells in C3H/HeN mice were more increased than in the C57BL/6 mice. The HPA-positive cells increased in the duodenum during early in-

fection, and they were somewhat increased in the jejunum and the ileum from week 3 P.I.

DISCUSSION

The immune sensitivity in host depends on the species of the host. Several species of hosts have different ability to remove an infecting parasite. There have been reports about the important roles of mast cells and goblet cells for worm expulsion (Chai et al., 1993; Fujino et al., 1998; Koyama et al., 2000).

Mast cells function differently for worm expulsion depending on the kind of host. It has been reported that mast cells played important roles in the mice infected with *Strongyloides ratti* and the goblet cells were associated with worm excretion in the mice infected with *N. braziliensis* and *Echinostoma trivolvis* (Mimori et al., 1982; Ishikawa et al., 1994). However, the mast cells and goblet cells in the rats infected with *Trichuris muris* or *Hymenolepis diminuta* had no effect on worm expulsion (Koyama et al., 2000). In the present study, we investigated whether the change in the number of mast cells and goblet cells was associated with the mouse strain and worm expulsion. Our results demonstrated that the kinetics of mast cells were different in the C3H/HeN mice and the C57BL/6 mice. Worm expulsion continued until week 3 P.I. in the C3H/HeN mice; however, it was sustained up to week 5 P.I. in the C57BL/6 mice. Mast cells in the duodenum of the C3H/HeN mice and the C57BL/6 mice reached a peak at week 3 P.I and week 2 P.I., respectively (123.7±6.3, 100.0±18.5, respectively) (Fig. 1). Mucosal mast cells had no statistical effect on worm expulsion. C3H/HeN mice with *E. hortense* infection were more sensitive than the C57BL/6 mice due to role of the mast cells in the local immune response.

Goblet cells in the intestine mucosa have stimulated considerable interest for investigating their role as protectors in *Nippostrongyls brazilensis* and *Echinostoma trivolvis* infection (Miller et al., 1981; Abe et al., 1992; Abe et al., 1993; Fujino et al., 1993). It has been reported that goblet cells grew excessively and mucin was secreted during some nematoda and trematoda infections (Garside et al., 1992; Ishikawa et al., 1997). The goblet cell hypertrophy and mucin hypersecretion were modulated by the Th2 immune response and both were associated with worm expulsion. (Miller et al., 1975; Carroll et al., 1984; Miller, 1987; Grencis et al., 1991; Garside et al., 1992; Fujino et al., 1993; Khan et al., 1995; Ishikawa et al., 1997; Fallon et al., 2000; Khan et al., 2001). In contrast to the number of goblet cells, worm expulsion was noted to be regulated by the alteration of goblet cell function through the modification of mucin's terminal sugar, which was detected by lectin histochemistry (Ishikawa et al., 1993; Ishikawa et al., 1994). In our experiments, the number of goblet cells in the duodenum of the C57BL/6 mice reached a maximum level in week 2 P.I. (169.3±8.2) and the number of goblet cells in the ileum peaked in week 3 P.I. (125.0±20.0). The number of goblet cells in the duodenum of the C3H/HeN mice reached a peak in week 2 P.I. (233.3±28.7) and that in the jejunum and ileum peaked in week 3 P.I. (135.3±4.5, 138.0±9.5) (Fig. 2). The goblet cells increased until week 6 P.I. and they existed in the duodenum, jejunum and ileum of both mouse strains. These results indicated that worm expulsion was associated with the goblet cell in both strains. However, the worm expulsion was not related with C57BL/6 mice as a sensitive strain, or with the C3H/HeN mice as a resistant strain. These conflicting results cannot be currently explained. Further studies are needed to examine the exact mechanism of worm expulsion and the species specificity.

We performed lectin histochemistry for the precise examination of the modified mucin. Lectin-binding goblet cells were significantly increased in *E. hortense*-infected group in comparison with the control group ($P<0.001$). The number of lectin-binding goblet cells in the duodenum of the C3H/HeN mice (155.7±8.7) was higher than that of the C57BL/6 mice (83.7±8.2). After week 3 P.I., the number lectin-binding goblet cells in the duodenum rapidly decreased; however, the number slowly declined in the jejunum and ileum. These results indicated that the goblet cells in the C57BL/6 mice and in the C3H/HeN mice may regulate worm expulsion through altering the mucin terminal sugar.

In summary, we demonstrated that the goblet cells were closely associated with worm expulsion in comparison with the mast cells. Lectin-binding goblet cells having an altered mucin terminal sugar may also play important roles in worm expulsion. Our results provide further evidence of the modulation of the immune responses in mice with a parasite infection. This study was supported by a grant from the Maeji Institute of Academic Research in the fiscal year 2004.

REFERENCES

Abe T, Sugaya H, Yoshimura K, Nawa Y. Induction of the expulsion of *Strongyloides ratti* and retention of *Nippostrongylus brasiliensis* in athymic nude mice by repetitive administration of recombinant IL-3. Immunology. 1992. 76: 10-14.

Abe T, Sugaya H, Yoshimura K. Diffrent susceptibility to the IL-3 induced protective effects between *Strongyloides ratti* and *Nippostrongylus brasiliensis* in C57BL/6 mice. Parasitol Immunol. 1993. 15: 643-645.

Barrett KE, Neva FA, Gam AA, Cicmanec J, London WT, Philips JM, Metcalfe DD. The immune response to nematode parasites of mast cell numbers and function during stongyloides stercoralis infections in nonhuman primates. Am J Trop Med Hyg. 1988. 38(3): 574-581.

Carroll SM, Mayrhofer G, Dawkins HJ, Grove DI. Kinetics of intestinal lamina propria mast cells, globule leucocytes, intraepithelial lymphocytes, goblet cells and eosinophils in murine strongyloidiasis. Int Arch Allergy Appl Immunol. 1984. 74: 311-317.

Chai JY, Kim TH, Kho WG, Chung SW, Hong ST, Lee SH. Mucosal mast cell responses to experomental *Metagonimus yokogawai* infection in rats. Korean J Parasitol. 1993. 31(2): 129-134.

Else KJ, Finkelman FD. Intestinal nematode parasites, cytokines and effector mechanisms. Int J Parasitol. 1998. 28: 1145-1158.

Fallon PG, Smith P, Richardson EJ, Jones FJ, Faulkner HC, Van Snick J, Renauld JC, Grencis RK, Dunne DW. Expression of interleukin-9 leads to Th2 cytokine-dominated responses and fatal enteropathy in mice with chronic *Schistosoma mansoni* infections. Infect Immun. 2000. 68: 6005-6011.

Fujino T, Fried B, Tada I. The expulsion of *Echinostoma trivolvis*: worm kinetics and intestinal cytopathology in conventional and congenitally athymic BALB/c mice. Parasitology. 1993. 106: 297-304.

Garside P, Grencis RK, Mowat AM. T lymphocyte dependent enteropathy in murine *Trichinella spiralis* infection. Parasite Immunol. 1992. 14: 217-225.

Grencis RK, Hultner L, Else KJ. Host protective immunity to *Trichinella spiralis* in mice: activation of Th cell subsets and lymphokine secretion in mice expressing different response phenotypes. Immunology. 1991. 74: 329-332

Harari Y, Russell DA, Castro GA. Anaphylaxis-mediated epithelial Cl-secretin and parasite rejection in rat intestine. J Immunol. 1987. 138(4): 1250-1255.

Ishikawa N, Horii Y, Nawa Y. Immune-mediated alteration of the terminal sugars of goblet cell mucins in the small intestine of *Nippostrongylus brasiliensis*-infected rats. Immunology. 1993. 78: 303-307.

Ishikawa N. Histochemical characteristics of the goblet cell mucins and their role in defence mechanisms against *Nippostrongylus brasiliensis* infection in the small intestine of mice. Parasite Immunol. 1994. 16: 649-654

Ishikawa N, Wakelin D, Mahida YR. Role of T helper 2 cells in intestinal goblet cell hyperplasia in mice infected with *Trichinella spiralis*. Gastroenterology. 1997. 113: 542-549.

Khan WI, Abe T, Ishikawa N, Nawa Y, Yoshimura K. Reduced amount of intestinal mucus by treatment with anti-CD4 antibody interferes with the spontaneous cure of *Nippostrongylus brasiliensis*-infection in mice. Parasite Immunol. 1995. 17: 485-491.

Khan WI, Vallance BA, Blennerhassett PA, Deng Y, Verdu EF, Matthaei KI, Collins SM. Critical role for signal transducer and activator of transcription factor 6 in mediating intestinal muscle hypercontractility and worm expulsion in *Trichinella spiralis*-infected mice. Infect Immun. 2001. 69: 838-844.

Khan WI, Richard M, Akiho H, Blennerhasset PA, Humphreys NE, Grencis RK, Van Snick J, Collins SM. Modulation of intestinal muscle contraction by interleukin-9 (IL-9) or IL-9 neutralization: correlation with worm expulsion in murine nematode infections. Infect Immun. 2003. 71: 2430-2438.

Koyama K, Ito Y. Mucosal mast cell responses are not required for protrction against infection with the murine nematode parasite *Trichuris muris*. Parasite Immunol. 2000. 22: 13-20.

Meslin JC, Fontaine N, Andrieux C. Variation of mucin distribution in the rat intestine, caecum and colon: effect of the bacterial flora. Comp Biochem Physiol A Mol Int Physiol. 1999. 123: 235-239.

Miler HRP, Huntley JF, Wallace GR. Immune expulsion and mucous trapping during the rapid expulsion of *Nippostrongylus brasiliensis* from primed rats. Immunology. 1981. 44: 419-429.

Miller HR, Nawa Y. *Nippostrongylus brasiliensis*: intestinal goblet-cell response in adoptively immunized rats. Exp Parasitol. 1979. 47: 81-90.

Miller HR. Gastrointestinal mucus, a medium for survival and for elimination of parasitic nematodes and protozoa. Parasitology. 1987. 94: Suppl, S77-100.

Mimori T, Nawa Y, Koremaga M, Tada I. *Strongyloides ratti*: mast

cell and goblet cell responses in the small intestine of infected rats. Exp Parasitol. 1982. 54: 366-370.

Onah DN, Nawa Y. Mucosal immunity against parasitic gastrointestinal nematodes. Korean J Parasitol. 2000. 38: 209-236.

Perdue MH, Marshall J, Masson S. Ion transport abnormalities in inflamed rat jejunum. Involvement of mast cells and nerves. Gastroenterology. 1990. 98(3): 561-567.