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Mucosal mast cell responses to experimental *Metagonimus yokogawai* infection in rats

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Abstract: Intestinal mucosal mast cell (MMC) responses were studied in rats experimentally infected with *Metagonimus yokogawai* (Digenea: Heterophyidae). Twenty Sprague-Dawley rats were fed each 2,500 metacercariae isolated from the sweetfish and sacrificed on the week 1, 2, 3 and 4 post-infection (PI). Recovery of worms was performed from the small intestine of each rat. To visualize the MMCs, duodenal and jejunal (upper, middle and lower) tissue sections were made and stained with alcian blue/safranine-O. The average worm recovery rates were 16.2% and 13.8% on the week 1 and week 2, respectively, but they decreased rapidly to 4.1% and 4.2% on the week 3 and week 4 PI, respectively, which indicate spontaneous worm expulsion after the week 2. The MMC number in the infected rats was, compared with uninfected controls, significantly increased in the whole small intestine, through the whole period of observation. The peak level of mastocytosis was observed on the week 3 PI. It is strongly suggested that MMCs might be involved in the expulsion process of flukes from the rat intestine.

Key words: *Metagonimus yokogawai*, intestinal fluke, mucosal mast cell (MMC), worm expulsion, rat

INTRODUCTION

Metagonimus yokogawai is one of the commonest and important intestinal flukes of humans in Korea (Chai and Lee, 1990), and many studies are needed to understand host-parasite relationships. However, no much information has been available on immunological aspects of this fluke infection including MMC responses of the host.

Intestinal mucosal mast cells (MMCs) tend to draw increasing interest because they are considered to be deeply involved in immunophysiological events at the mucosal sites (Lee *et al.*, 1986; Castro, 1989). Increase in the number of MMCs, for instance, has been reported in various intestinal diseases including parasitic infections such as *Nippostrongylus brasiliensis* (Miller and Jarrett, 1971), *Hymenolepis diminuta* (Andreassen *et al.*, 1978), *Strongyloides ratti* (Mimori *et al.*, 1982), *Trichinella spiralis* (Woodbury *et al.*, 1984), and *Fibricola seoulensis* (Kho *et al.*, 1990). Besides increases in the number, MMCs

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were shown to be functionally active during the expulsion phase of infection with *N. brasiliensis* or *T. spiralis*, with significantly increased levels of serum mast cell protease II (Woodbury *et al.*, 1984).

In this respect, MMCs have been strongly suggested to play an important role in the expulsion of intestinal helminths from the host intestine. This study aimed to observe MMC responses in rats experimentally infected with *M. yokogawai*.

MATERIALS AND METHODS

Twenty Sprague-Dawley rats (males) weighing 50-70 g were infected orally each with 2,500 metacercariae of *M. yokogawai*, isolated by peptic digestion of 110 sweetfish caught at Hadong-gun, Kyongsangnam-do. The rats (5 each) were sacrificed on the week 1, week 2, week 3 and week 4 post-infection (PI). Six uninfected rats were used for controls.

The small intestines of infected rats were resected, and opened longitudinally for recovery of the worms. After gentle shaking of the intestine soaked in cold saline (4°C) for 2 hrs, the freed worms were counted under a dissecting microscope.

To observe the MMCs, the intestinal segments, about 1.5 cm in each length, were taken from four portions of the small intestine: 5 cm, 15 cm, and 45 cm posterior to the pylorus, and 1 cm anterior to the ileo-cecal junction; designated as the duodenum, upper, middle, and lower parts of the jejunum, respectively. The segments were fixed in Carnoy's solution for 2 hrs, embedded in paraffin, and sectioned in 4 micron thickness. According to the method described by Strobel *et al.* (1981), the sectioned samples were stained with 1% alcian blue (pH 0.3) and counterstained with 0.5% safranine-O.

The MMCs were counted in well-oriented cross sections (one representative slide per each sample) of the small intestine. The counts were performed using a microscope equipped with eyepiece graticule (eyepiece × 10, objective × 10). The edge of the graticule was oriented along the muscularis mucosae at the base of the crypt. The total number of MMCs in the field covered by the square of the graticule (500 × 500 microns; area 0.25 mm^2) was counted. For each sectioned specimen, a minimum of 3 fields were counted and thus total 15 MMC counts per each group (5 rats) were obtained. Values were expressed as average MMCs (/0.25 mm^2) and standard deviation. Results of various groups were analyzed by Student's *t* test.

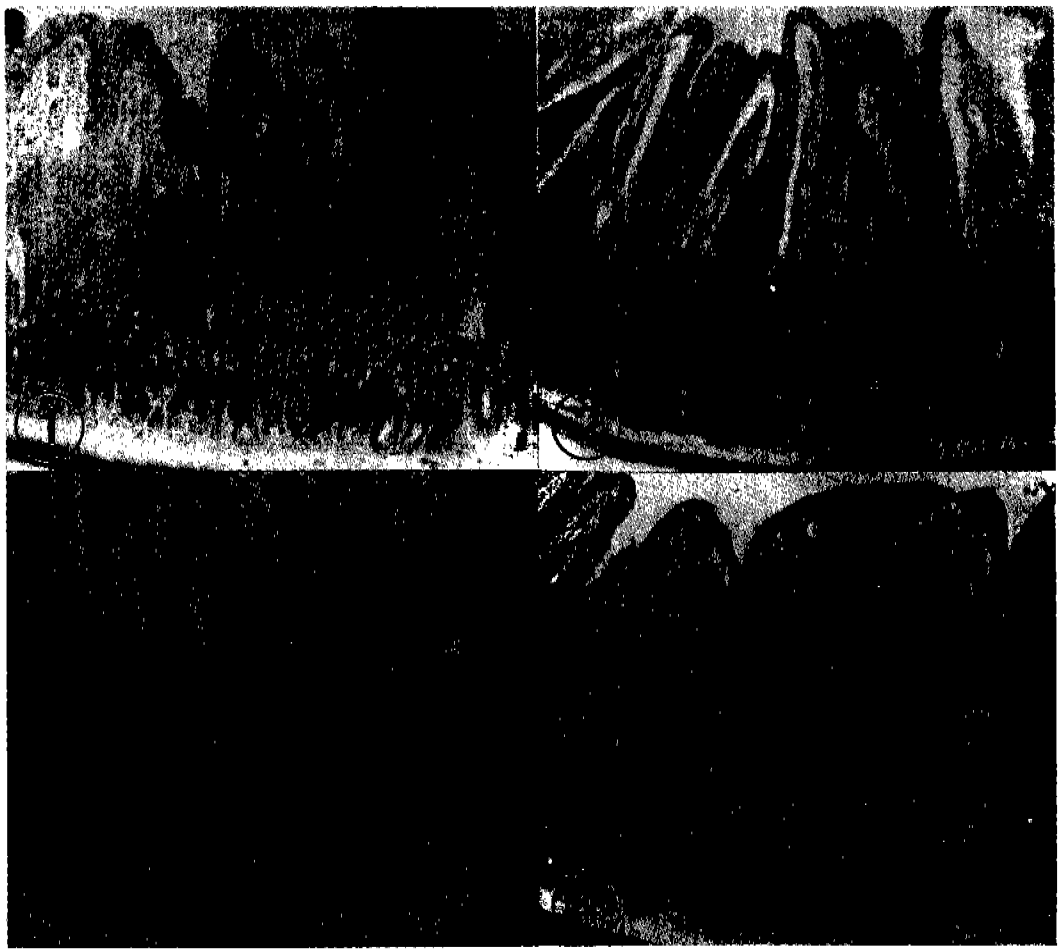
RESULTS

1. Worm recovery rate from the rats

On the week 1 PI, a total of 2,024 adult worms of *M. yokogawai* were recovered from the small intestine of 5 rats given a total number of 12,500 metacercariae (worm recovery rate; 16.2%), and on the week 2 PI, a total of 1,726 worms were recovered (worm recovery rate; 13.8%). The worm recovery rate decreased rapidly to 4.1% (513 worms) and 4.2% (523 worms) on the week 3 and week 4 PI, respectively, which means spontaneous expulsion of flukes after the week 2 PI.

2. MMCs in the small intestine of rats

Most of the MMCs were found in the lamina propria of the small intestine in the controls (Fig. 1), as well as in the infected rats (Figs. 2-4). In the control rats, the MMC numbers (/0.25 mm^2) were not significantly different by locations in the intestine; the duodenum, upper, middle and lower portions of the jejunum (22.9 ± 3.1, 23.8 ± 5.1, 23.1 ± 4.6, 24.4 ± 2.8, respectively). On the week 1 after the infection with *M. yokogawai*, the number of MMCs significantly ($p < 0.01$) increased (Figs. 2 & 5) in all of the 4 portions of the small intestine (31.6 ± 4.0, 33.4 ± 6.0, 36.1 ± 4.7, 36.8 ± 3.9, respectively). The number further increased on the week 2 (36.4 ± 4.7, 34.2 ± 5.0, 38.4 ± 5.4, 35.8 ± 5.5), and made peak values (49.0 ± 6.4, 42.8 ± 4.6, 46.6 ± 8.4, 54.3 ± 7.0) on the week 3 PI (Figs. 3 & 5). Then the MMC number decreased(39.5 ± 4.5, 40.6 ± 4.2, 37.6 ± 3.5, 39.5 ± 4.6) on the week 4 PI (Figs. 4 & 5). Significant reverse correlations were recognized between the MMC numbers and the worm recovery rate (Fig. 5). No regional difference was noted in the number of MMCs in infected rats (Fig. 5).



Figs. 1-4. Sections of the duodenum of rats infected with *M. yokogawai* in comparison with control group. Blue spots (arrows) in the lamina propria and epithelial layer are the mucosal mast cells (MMCs). **1.** uninfected control group, showing only a small number of MMCs. **2.** infected rat, week 1 PI, a significant increase in the number of MMCs is recognizable. **3.** infected rat, week 3 PI, showing markedly increased number (peak level) of MMCs. **4.** infected rat, week 4 PI, with decreased number of MMCs compared with week 3 PI. Alcian blue (pH 0.3)/safranine-0 stained, × 100.

DISCUSSION

The clinical symptoms due to *M. yokogawai* infection are generally mild but some of the infected persons may suffer from severe diarrhea and abdominal pain (Seo *et al.*, 1971). The worms are known to dwell rather scatteredly in the upper, middle and lower portions of the small intestine in experimental animals (Chai, 1979; Kang *et al.*, 1983). In rats infected with *M. yokogawai*, villous atrophy and crypt hyperplasia with severe inflammatory changes in the stroma of villi were observed (Chai, 1979). However, there have been few reports on mucosal immune responses of the host to this fluke infection.

The present study showed that mucosal mastocytosis occurred in the small intestine of rats experimentally infected with *M. yokogawai*. It was, though milder in degree, very similar to the mucosal mastocytosis reported in the rats infected with *Fibricola seoulensis* (Kho *et al.*, 1990), which is another intestinal fluke eliciting severe mucosal changes and destruction in host animals (Seo, 1990). These observations together suggest that MMCs might be important in mucosal

defense mechanisms of the host to fluke infections.

In this study, increases in the number of MMCs were observed in all of the 4 different portions of the small intestine examined. This was different from *F. seoulensis* infection, where mastocytosis was observed only in the upper part of the small intestine (Kho *et al.*, 1990). Such a difference in local preponderance of mastocytosis seems to be related with the habitat or ecological niche of the flukes as well as the different worm distribution patterns between the two kinds of flukes. It is well known that *M. yokogawai* are distributed well over the whole small intestine (Kang *et al.*, 1983), whereas *F. seoulensis* dwell mostly in the duodenum (Hong, 1982). This tendency was also true in intestinal nematode infections; mucosal mastocytosis was restricted to the site of worm infection (Mimori *et al.*, 1982; Guy-Grand *et al.*, 1984).

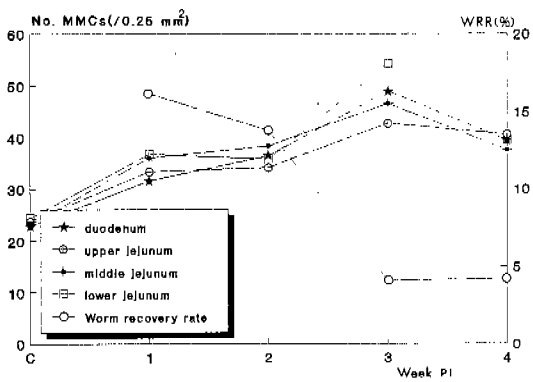


Fig. 5. Correlations between the worm recovery rate (WRR) and number of MMCs (No. MMCs) in different locations of the small intestine of the rats experimentally infected with *M. yokogawai*.

The maximal mastocytosis was observed on the week 3 PI when the worm recovery rate decreased abruptly. Similar results were also observed in *F. seoulensis* infection (Kho *et al.*, 1990). Although no functional studies were performed in this study, the correlation between the changing patterns of MMC numbers and decrease of the worm recovery rate strongly suggests that MMCs play a significant role in worm expulsion from the rat intestine.

Although more precise role of MMCs in intestinal helminth infections is yet to be further defined, the possible way they participate in the process of worm expulsion has been proposed by several groups of investigators (Woodbury *et al.*, 1984; Moqbel and MacDonald, 1990). According to them, it is well known that the activated MMCs release and generate many kinds of potent mediators. They include pre-formed mediators such as histamine, eosinophil chemotactic factor-A, neutrophil chemotactic factor or proteases, and newly formed mediators such as leukotrienes, platelet-activating factor or prostaglandins (Moqbel and MacDonald, 1990). The exact function of each mediator in enhancing the inflammatory process and the extent of their contribution to worm damage and expulsion is still obscure. However, it was

Table 1. Mucosal mast cell (MMC) numbers in the small intestine of rats experimentally infected with *M. yokogawai*

Group	No. of MMCs per 0.25 mm² in the mucosa of			
	duodenum	jejunum		
		upper	middle	lower
Control	22.9 ± 3.1[a]	23.8 ± 5.1	23.1 ± 4.6	24.4 ± 2.8
Infected				
week 1	31.6 ± 4.0	33.4 ± 6.0	36.1 ± 4.7	36.8 ± 3.9
week 2	36.4 ± 4.7	34.2 ± 5.0	38.4 ±5.4	35.8 ± 5.5
week 3	49.0 ± 6.4	42.8 ± 4.6	46.6 ± 8.4	54.3 ± 7.0
week 4	39.5 ± 4.5	40.6 ± 4.2	37.6 ± 3.5	39.5 ± 4.6

[a]Values are expressed as the mean ± S.D. of 5 (infected) or 6 (control) rats.

strongly suggested that a combination of mediators are involved, both directly by their spasmogenic and vasoactive properties, and indirectly by recruiting and activating other cells in the inflamed tissues (Moqbel and MacDonald, 1990). They further suggested that the interaction of MMCs and IgE play a role in the mucosal anaphylaxis and elimination of intestinal worms.

These kinds of events may finally lead to severe pathophysiological changes in the intestinal environment which are deleterious to the parasite survival. In metagonimiasis of rats, for example, the occurrence of severe pathological changes in the mucosa (Chai, 1979) is suggested, in turn, to give a harmful effect for the establishment of the flukes in the intestine. MMCs may deeply involve in these pathologic changes and may participate in the worm expulsion. Further studies on the nature and functions of MMCs in the expulsion of *M. yokogawai* from the gut are highly recommended.

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=국문초록=

요꼬가와흡충 감염에 대한 흰쥐 장 점막 비만세포의 반응

서울대학교 의과대학 기생충학교실 및 풍토병연구소[1], 인하대학교 의과대학 소아과학교실[2]

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장 점막 비만세포(mucosal mast cells)는 여러 장 질환을 비롯하여 기생충 감염증 등에서 중가함이 잘 알려져 있으나, 우리 나라에 흔한 인체 장흡충류인 요꼬가와흡충 감염에 있어서는 연구가 없었다. 이 연구는 흰쥐의 실험적 요꼬가와흡충증을 모델로 하여 감염 후 충체가 자연 치유되는 약 4주 동안 충체 회수율의 변화 및 장 점막 비만세포 수의 변화를 경시적으로 관찰한 것이다. Sprague-Dawley계 흰쥐 1마리당 요꼬가와흡충 피낭유충 2,500개씩을 총 20마리에 감염시켰고(비감염 흰쥐 6마리를 대조군으로 둠), 감염 1, 2, 3, 4주 후에 각각 5마리씩 희생시켜 대조군과 비교하였다. 장 점막 비만세포는 흰쥐의 십이지장, 공장 상부, 중부 및 하부로부터 조직을 채취하여 Carnoy액에 고정한 후 절편을 만들고 alcian blue(pH 0.3)/safranine-O 염색을 하여 관찰하였다. 연구 결과, 충체 회수율은 감염 1주 및 2주에 평균 16.2% 및 13.8%, 3주 및 4주에 각각 4.1% 및 4.2%로 나타나, 감염 2주까지 비슷한 수준을 유지하다가 3-4주에 급격히 감소하는 양상을 보여 주었다. 비만세포의 수는 감염 1주부터 대조군에 비해 유의한 증가를 보였고, 3주에 가장 높은 수치를 보이다가 4주째에 약간 감소하였다. 십이지장, 공장 상부, 중부 및 하부의 부위에 따른 비만세포 수의 차이는 없었다. 충체 회수율과 비만세포 수의 변화 양상을 비교해 볼 때, 비만세포가 충체 회수율이 급격히 감소하는 시기에 맞추어 가장 높은 수치를 보이므로 충체 배출과 관계되는 어떤 중요한 역할을 하고 있을 것으로 추측되었다.

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