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Dermal mast cell responses in *Paragonimus westermani*-infected mice

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Abstract: This study was carried out to determine whether dermal mast cell responses to *Paragonimus westermani* in an abnormal host, the mouse, were dependent on the site of metacercarial inoculation. In mice during subcutaneous infection, the number of dermal mast cells were increased significantly ($p<0.05$) at the first week (38.3/mm^2) and then persisted at a high level until the sixth week (45.2/mm^2) of infection compared with PBS-injected (control) mice (range: 19.4-25.1/mm^2). In mice during oral infection, the number of dermal mast cells were increased significantly ($p<0.05$) at two weeks (33.5/mm^2) after infection and remained at these levels thereafter compared with non-infected (control) mice (range: 17.4-22.3/mm^2). In mice both during subcutaneous and oral infection, the recruited dermal mast cells showed extensive degranulation at the second week (68.4% and 60.7%, respectively), reached a peak at the third week (81.4%, and 92.1%, respectively) and then declined slightly thereafter. By contrast, in both control mice, about 10% of dermal mast cells were degranulated. In conclusion, this study suggests that dermal mast cell responses to *P. westermani* in mice are dependent on cutaneous sensitization by larval excretory-secretory antigens, irrespective of infection route.

Key words: *Paragonimus westermani*, mast cell responses, mice

INTRODUCTION

Although it has been well known that recruitment of mast cells is a common feature of helminthic infections in most host species, the timing and degree of mast cell infiltration can vary between species and with the intensity and chronicity of infection. Infection with intestinal helminths in rats and mice, such as *Trichinella spiralis*, *Strongyloides ratti*, *Metagonimus yokogawai* and *Hymenolepis nana*, is associated with early, very intense, recruitment of intestinal mast cells (Mimori *et al.*, 1982; Woodbury *et al.*, 1984; Chai *et al.*, 1993; Watanabe *et al.*, 1994). In contrast to infection with intestinal helminths, infection with tissue helminth like *Schistosoma mansoni* in mice induced low grade intestinal mastocytosis over many weeks (Weinstock and Boros, 1983). Therefore, lung fluke, *Paragonimus westermani*, are likely to induce tissue mast cell responses as worms migrate within the host until they reach their final location. Mice are abnormal hosts for *P. westermani*, and the most of worms can survive for more than 20 weeks in mouse muscles without marked growth and development (Min *et al.*, 1993). There have been few experimental studies of the interaction between dermal mast cell responses and the life cycle of *P. westermani* in mice. And also, little is known whether dermal mast cell responses to *P. westermani* in mice are dependent on the site of metacercari-

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al inoculation.

In this study, mast cell numbers and extent of mast cell degranulation at skin during subcutaneous or oral infection with *P. westermani* in BALB/c mice were examined to determine whether dermal mast cell responses are influenced by the mode of infection route.

MATERIALS AND METHODS

Parasites and experimental animals

Metacercariae of *P. westermani* were separated from crayfish, *Cambaroides similis*, collected at Wando-gun, Chollanam-do, Korea. Four to five week-old BALB/c female mice were purchased from Korea Experimental Animal Center. Mice were infected as follows: 1) subcutaneous infection: each mouse was infected by inoculating 10 metacercariae of *P. westermani* subcutaneously into back skin. 2) oral infection: each mouse was infected orally with 10 metacercariae. Age-matched mice were used as PBS-injected (control) and non-infected (control) groups.

Mast cell number and extent of mast cell degranulation

Mice were killed on weeks 1, 2, 3, 4 and 6 post infection. A strip of tissue from back skin, about 2 × 2 cm in size, was fixed in Carnoy's solution for 2 hrs, embedded in paraffin and sectioned in 5 μm thickness. According to the method described by Im *et al.* (1975), the sectioned tissue slides were stained with 0.4% toluidine blue. Toluidine blue-stained mast cells were counted under low magnification (× 100) of a light microscope equipped with a eyepiece graticule (× 10 eyepiece and × 10 objective) in order to insure that overlap and double counting did not occur. The edge of graticule was oriented along the muscularis mucosa at the top of the subcutaneous layer. The total number of mast cells in the field covered by the square of the graticule [(average height of dermis × length) (0.5 mm × 1 mm; area 0.5 mm^2)] were counted by a single observer. The height of dermis included in the field varies from 80-100%, depending on shrinkage of the specimen. One each slide, five fields were counted and data were expressed as the average number of dermal mast cells (/mm^2) and standard deviation. It was also determined what proportion of one hundred dermal mast cells showed evidence of degranulation. The mast cells were counted in high power fields (× 400) as degranulated if more than ten granules were present free of the cell mass. Scattered mass of granules without a cell nucleus were not counted.

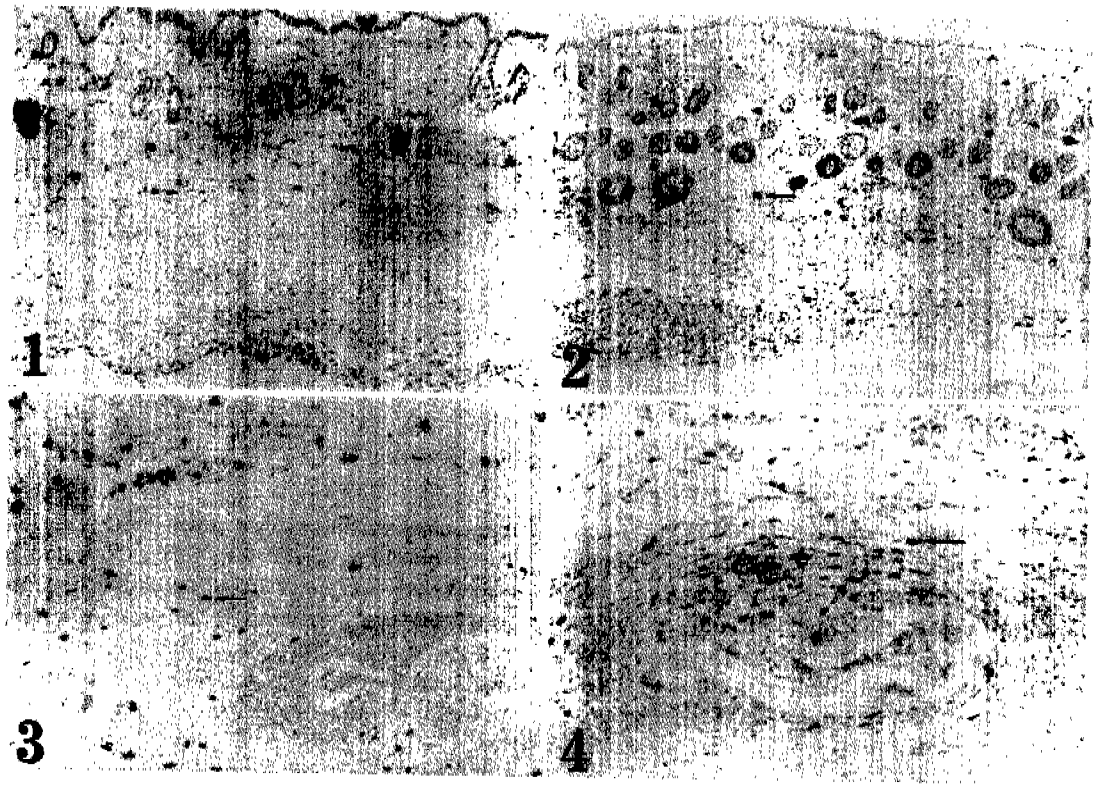
Statistical analysis

Differences in mast cell number/mm^2 of dermis and percentage of the mast cells that exhibited degranulation between the groups were tested for statistical significance ($p<0.05$) by the Student's t-test.

RESULTS

Dermal mast cell numbers during subcutaneous infection with *P. westermani* in mice increased significantly ($p<0.05$) at week 1 (38.3/mm^2) and peaked at week 3 (46.5/mm^2), and then remained at these levels until the end of sixth week (45.2/mm^2) of infection compared with PBS-injected (control) mice (Table 1, Figs. 1 & 2). In addition, many mast cells during subcutaneous infection are largely confined to dermal layer neighbors in the cyst of worm of subcutaneous layer, and the mast cells recruited to granulomatous lesion surrounding cyst of worms in subcutaneous layer (Figs. 3 & 4). Dermal mast cell numbers during oral infection were increased significantly ($p<0.05$) at two weeks (33.5/mm^2) and peaked at three weeks (41.4/mm^2), and then persisted at a high level until the sixth week of infection (38.4/mm^2) compared with non-infected (control) mice (Table 1).

In mice both during subcutaneous and oral infection, many mast cells at dermal layer exhibited fully extensive degranulation, especially at three weeks after infection (Figs. 5 & 6). The mean percentages of degranulated dermal mast cells in both during subcutaneous and oral infection were increased significantly at two weeks (68.4% and 60.7%, respectively) after infection and peaked at three weeks (81.3% and 92.1%, respectively), and then dedined slightly at the sixth week of infection (69.4% and 74.1%, respectively) (Table 1). By contrast, in PBS-injected (control) and non-infected (control) mice, only about 10% of the mast cells exhibited histologic evidence of



Figs. 1-4. Mast cells at the skin in mice during subcutaneous infection with *P. westermani* compared with PBS-injected (control) mice. Blue spots (arrows) in the dermis and subcutaneous layer are the mast cells. **1.** PBS-injected (control) mice, showing only small number of dermal mast cells, × 100. **2.** In mice during subcutaneous infection, week 1 PI, showing a significant increase in the number of mast cells of dermis and subcutaneous layer, × 100. **3.** In mice during subcutaneous infection, week 2 PI, many mast cells are distributed adjacent to a cyst of worm, × 100. **4.** In mice during subcutaneous infection, week 3 PI, degranulated mast cells with open-faced nuclei are shown around a cyst of worm in subcutaneous layer, × 200. Toluidine blue staining, pH 0.5.

extensive degranulation during the entire course of experiment (Table 1).

DISCUSSION

This study shows that *P. westermani* induces dermal mastocytosis and extensive degranulation of the recruited dermal mast cells in mice both during subcutaneous and oral infection. The minor difference was that the recruitment of the mast cells during subcutaneous infection was earlier and more intense than during oral infection. And also, mast cells during subcutaneous infection are largely confined to dermal layer neighbors in the cyst of worm in subcutaneous layer and many mast cells also selectively recruited to subcutaneous layer around the cyst of worm. These results indicate that recruitment of dermal mast cells appear to be partly dependent on the degree of local stimulation of excretory-secretory products from infecting parasites. Similar results were shown in other helminthic infections. In rats infected with *Neodiplostomum seoulense* which mainly dwells in duodenum, marked increase in the number of mucosal mast cells (MMC) was only observed in the upper part of small intestine (Kho *et al.*, 1990). There was no significant increase of MMC at the lower part of the small intestine. And also, hepatic recuitment of mast cells occurred in rats but not mice infected with *S.*

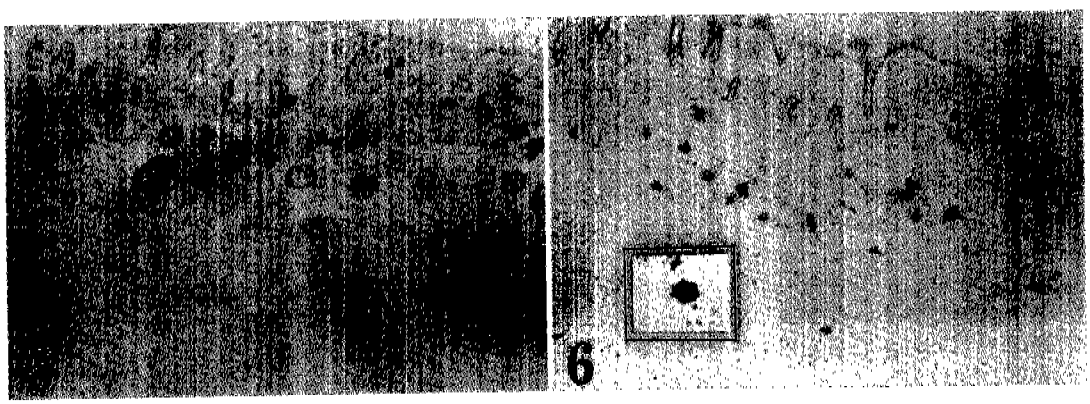
Table 1. Dermal mast cell number and extent of mast cell degranulation during *P. westermani* infection in BALB/c mice

Weeks after infection	Mast cell number per 1 mm^2				Degranulation of mast cell (%)			
	SC. Inf.[a]	PBS-inj.[b]	O. Inf.[c]	Non-inf.[d]	SC. Inf.	PBS-inj.	O. Inf.	Non-inf.
1	38.3 ± 4.1[e]	25.1 ± 2.4	26.0 ± 3.3	22.3 ± 2.7	19 ± 4	10 ± 2	14 ± 3	6 ± 1
2	45.5 ± 7.9[e]	22.0 ± 3.1	33.5 ± 4.2[f]	20.8 ± 2.1	68 ± 9[e]	12 ± 2	65 ± 4[f]	10 ± 2
3	46.7 ± 8.0[e]	24.6 ± 2.7	41.4 ± 4.8[f]	20.2 ± 3.9	81 ± 10[e]	9 ± 1	92 ± 2[f]	9 ± 2
4	38.4 ± 5.5[e]	19.4 ± 2.8	36.8 ± 5.6[f]	19.7 ± 2.4	78 ± 5[e]	13 ± 2	74 ± 6[f]	8 ± 1
6	45.2 ± 7.8[e]	22.1 ± 3.2	38.4 ± 3.2[f]	17.4 ± 3.3	69 ± 8[e]	11 ± 2	74 ± 9[f]	11 ± 2

[a]Mice (n = 5) are infected subcutaneously with 10 metacercariae.
[b]Mice (n = 3) are injected subcutaneously with sterile PBS.
[c]Mice (n = 5) are infected orally with 10 metacercariae.
[d]Non-infected mice (n = 3).
[e]Student's t-test ($p<0.05$) *vs* PBS-injected (control) mice.
[f]Student's t-test ($p<0.05$) *vs* non-infected (control) mice.
Each value represents mean ± SD.

mansoni (Miller *et al.*, 1994). Thus the mast cell recruitment in tissue helminth infections are closely related to the location where the parasite lives in the host. Therefore, dermal mastocytosis in mice during oral infection like subcutaneous infection may be due to cutaneous stimulation by larval excretory-secretory antigens because the most of worms in mice infected orally with *P. westermani* can survive in superficial muscles in the skin.

If mast cells are degranulated in parasitic infections, it must be preceded by production of the parasite-specific IgE and its binding to FcεRIs on the surface of mast cell. In the present study, not only during oral infection but also during subcutaneous infection, dermal mast cells exhibited extensive degranulation. These result suggests that IgE production *in vivo* are irrepective of infection route, because subcutaneously inoculated metacercariae normally excyst and migrate *in vivo*. The evidence is that any worms are not found within metacercarial cysts of subcutaneous layer. This finding also means that *P. westermani* might infect hosts through the wounds on the skin. However, the time course of dermal mast cell degranulation in this study were not consistent with that of the parasite-specific IgE in mice infected with *P. westermani* previously reported by Shin and Min (1997). They reported that the parasite-specific IgE were increased significantly at the first sixth week of



Figs. 5-6. In mice during subcutaneous (**5**) and oral infection (**6**) with *P. westermani*, respectively, week 3 PI, many mast cells are degranulated, × 100. A rectangle indicates higher magnification (× 200) of fully extensive degranulated mast cells. The nucleus of mast cell is heavily stained and numerous cytoplamic granules are extruded from the cell. Toluidine blue staining, pH 0.5.

infection. This discrepancy may be understood by reason that the parasite-specific IgE measured by ELISA in that study are not mast cell-bound IgE but serum IgE. Since the measurement of mast cell-bound IgE *in vivo* would provide a more realistic indication of mast cell degranulation in rats infected with *S. mansoni* (Cuttis and Wilson, 1997). An another reason for time discrepancy between dermal mast cell degranulation and parasite-specific IgE levels in sera during *P. westermani* infection may be that very low levels of the parasite-specific IgE during the early infection could not be detected by conventional ELISA. Because the parasite-specific IgE level represents a very small portion of total IgE (Rousseaux-Provost *et al.*, 1978), and serum IgE detection is also compounded by the action of specific blocking antibody; for example, IgG subclasses compete with IgE for antigen bindings (Boctor and Peter, 1987).

There is a debate about mastocytosis in relation to worm expulsion. Furthermore, systemic infection with some tissue-dwelling helminths, where the parasites can survive for long periods, can be associated with mastocytosis (Lindsay and Williams, 1985; Chernin *et al.*, 1988). Under these circumstances, mast cells presumably have no protective role. Conversely, the immune elimination of nematodes from mast cell-deficient rats occur in the absence of mast cell responses (Arizono *et al.*, 1993). Taken together, these studies suggest that mast cells are involved in but are not central to the expulsion process. Indeed, mast cells may have a central role in initial induction of a Th2 response (Romagnani, 1992) and mast cell-derived mediators, such as histamine, have been shown to down-regulate the production of Th1 cytokines such as IL-2 and IFN-γ (Falus and Meretey, 1992). In mice infected with *P. westermani*, Th2 cytokine responses predominated during the infection (Shin and Min, 1996). Therefore, further studies are needed to know how mast cells modulate Th2 immune responses in *P. westermani* infection.

This study showed that *P. westermani* induced dermal mastocytosis and extensive degranulation of dermal mast cells in mice both during subcutaneous and oral infection, although the recruitment of dermal mast cells during subcutaneous infection was earlier and more intense than during oral infection. In conclusion, this study suggests that dermal mast cell responses to *P. westermani* in mice are dependent on cutaneous sensitization by larval excretory-secretory antigens, irrespective of infection route.

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= 초록 =

폐흡충 감염에 대한 마우스 진피 내 비만세포의 반응

신명헌

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폐흡충 감염시 일어나는 비만세포 (mast cell)의 반응이 감염경로에 따라 어떠한 영향을 미치는지 알아보고자 비호적숙주인 마우스에 폐흡충의 피낭유충 10개를 피하 또는 경구 감염시킨 후 감염 시기별로 진피 내에 동원되는 비만세포의 숫적 변동 및 탈과립률 (%)을 경시적으로 관찰하였다. 피하로 감염시킨 마우스는 감염 1주 (38.3/mm^2)부터 진피 내 비만세포의 수가 PBS 주입군 (대조군) (범위: 19.4-25.1/mm^2)에 비해 유의한 수준 ($p<0.05$)으로 증가하기 시작하여 전 실험기간 동안 증가하였다. 이때 비만세포는 충체의 낭 (cyst) 주위의 피하층에도 집중적으로 침윤되어 있었다. 경구로 감염시킨 마우스는 감염 2주 (33.5/mm^2)부터 진피 내 비만세포의 수가 비감염군 (대조군) (범위: 17.4-22.3/mm^2)에 비해 유의한 수준 ($p<0.05$)으로 증가하기 시작하여 전 실험기간인 감염 6주 (38.4/mm^2)까지 높게 유지되었다. 진피 내에 동원된 비만세포의 평균 탈과립률 (%)은 피하 감염 및 경구 감염군 모두 감염 2주부터 6주까지 60% 이상을 보인 반면 PBS 주입군 (대조군) 및 비감염군 (대조군)에 있어서는 전 실험기간 동안 10% 정도의 탈과립률이 관찰되었다. 이상의 결과로 보아 폐흡충을 마우스에 감염시켰을 때 일어나는 진피 내 비만세포 반응은 감염경로와는 상관없이 충체에서 분비되는 배설-분비 항원의 자극과 깊은 관계가 있음을 알 수 있었다.

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