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## LONGEVITY AND FECUNDITY OF *TRICHINELLA SPIRALIS* IN MAST CELL-DEFICIENT SI/SI<sup>d</sup> MICE

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Response to *Trichinella spiralis* infection in genetically mast cell-deficient SI/SI<sup>d</sup> mice was studied. Very few or no subepithelial mast cells (SMC) and globule leucocytes (GL) were observed in WCB6F<sub>1</sub>-SI/SI<sup>d</sup> mice in the primary and tertiary infections with *T. spiralis*. While marked increases of these two cell types were seen in the primary and tertiary infections in their normal littermates. In both primary and tertiary infections delayed expulsion of adult worms from the intestine was seen in SI/SI<sup>d</sup> mice as compared with that in the normal littermates. In SI/SI<sup>d</sup> mice the expulsion of worms occurred only slightly faster in the tertiary infection than in the primary infection, whereas in the normal littermates, the expulsion was remarkably faster in the former than in the latter. No difference was noted in the number of muscle larvae recovered from SI/SI<sup>d</sup> mice and their littermates after intravenous injection of newborn larvae, but greater number of muscle larvae was recovered from SI/SI<sup>d</sup> mice after oral inoculation of infective muscle larvae. Adult worms obtained from SI/SI<sup>d</sup> mice showed greater fecundity *in vitro* than those from the normal littermates. In both SI/SI<sup>d</sup> mice and their normal littermates, no significant difference was noted in the production of specific antibodies, as shown by the indirect hemagglutination serum titers and the IgE titers measured by passive cutaneous anaphylaxis reaction. These results suggest a certain positive participation of SMC and GL in the resistance to intestinal phase of *T. spiralis* infection.

Key words: mucosal mast cell, globule leucocyte, SI/SI<sup>d</sup> mouse, *Trichinella spiralis*, worm expulsion.

### INTRODUCTION

Local cell components are important in the induction of expulsion of *Trichinella spiralis*.<sup>1)</sup> Subepithelial mast cells (SMC) and intraepithelially located globule leucocytes (GL) in the intestine of various animal species have been considered to play important roles in the expulsion of intestinal helminths, because infection with certain intestinal helminths lead to the marked accumulation of these cell types in the

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intestinal mucosa.<sup>7)</sup> But the function of GL and SMC in the expulsion of helminth is still not clear.<sup>22)</sup> The precursor cells of these two cell types are derived from the bone marrow.<sup>2,23)</sup> Some workers believed that GL is probably derived from SMC, so that these two cell types are generally called mucosal mast cells (MMC),<sup>18)</sup> but others claimed that GL may be independent of SMC and is a cell *sui generis*.<sup>30)</sup>

Congenitally athymic (nude) mice which showed the very few MMC accumulation in response to infection and failed to expel adult *T. spiralis* from the intestine, were repaired of both defects by thymus cells or thymus gland grafts transplantation.<sup>29)</sup> But the thymus was not always required in SMC and GL proliferation. Some experiments on infection with *Nippostrongylus brasiliensis* in genetically connective tissue mast cell-deficient W/W<sup>v</sup> mice were carried out and no increase in the number of MMC in the intestine was observed.<sup>16,31)</sup> We reported that the expulsion of *T. spiralis* was also delayed in W/W<sup>v</sup> mice.<sup>9)</sup>

The Sl/Sl<sup>d</sup> mouse has genetically determined macrocytic anemia which appears to be due to a tissue defect which prevents normal haematopoietic cells from proliferating.<sup>12)</sup> In adult W/W<sup>v</sup> and Sl/Sl<sup>d</sup> mice connective tissue mast cells were less than 1% of mast cells observed in congenic +/+ mice. Immunological defects in Sl/Sl<sup>d</sup> mice were not found.<sup>15)</sup> Therefore, the use of Sl/Sl<sup>d</sup> mice as well as W/W<sup>v</sup> mice is advantageous in the determination of the relative importance of MMC to the expulsion of intestinal parasites.

In order to assess the relevance of MMC to the resistance to *T. spiralis* infection, kinetics of SMC and GL, the expulsion patterns of adult worms from the intestine, muscle larval recovery, *in vitro* fecundity of adult worms and serum titers of antibodies against muscle larval antigen (measured by IHA and PCA) were examined using Sl/Sl<sup>d</sup> mice.

## MATERIALS AND METHODS

### *Mice*

Male WCB6F<sub>1</sub>-Sl/Sl<sup>d</sup> (WC-Sl/+ × C57BL/6J-Sl<sup>d</sup>/+) mice and their normal male littermates (Sl/+, Sl<sup>d</sup>/+, +/+) were obtained from the Jackson Laboratory, Bar Harbor, Maine, USA. Mice used in this study were more than 100 days old.

### *Parasite*

The strain of *T. spiralis* used was originally isolated in 1968 from a polar bear, *Thalarctos maritimus*, at Maruyama Zoo, Sapporo, Japan and was maintained in our laboratory.<sup>25)</sup> Infective muscle larvae used for oral infection were obtained from infected mice after digestion with artificial gastric juice (0.5% pepsin: Difco 1:10,000-0.5% HCl) at 37°C for 2 hours at a concentration of about 20 ml of gastric juice to 1g of the carcass. Mice were orally inoculated with infective muscle larvae suspended in saline, under ether-anaesthetized condition. Newborn larvae (NBL)

used for intravenous injection were obtained from adult *T. spiralis* harvested from the small intestine of Wistar rats 6 days after infection.<sup>11)</sup> These adults were placed in Hank's solution (Nissui) containing a penicillin/streptomycin mixture (50 unit/ml, 50mg/ml), and incubated for 8 hours at 37°C. The newly laid larvae and the adults were passed through a 200  $\mu$ m mesh sieve in order to separate them and NBL were suspended in Hank's solution. Mice were injected intravenously with NBL in 0.5ml Hank's solution.

#### *Primary and tertiary infection*

In order to examine the course of primary infection, each mouse was infected orally with 400 infective muscle larvae, and to examine the course of tertiary infection, two times of infection with 100 infective muscle larvae were carried out 7 and 3 weeks before the challenge infection with 400 infective muscle larvae.

#### *Adult worm counts*

The small intestine and the cecum of infected mice were removed and starting 1/3 of the whole small intestine distal to the pyloric sphincter of the stomach, 2-cm piece of small intestine was removed for the histological examination. The remaining intestine was stored at -40°C. To count the worm, the intestine was thawed, and split longitudinally in a petri dish filled with saline added with sodium hypochlorite solution. Under a dissection microscope (at  $\times 15$  magnification), worms were collected from the both intestinal wall and contents and were counted.

#### *Muscle larval recovery*

Five SI/SI<sup>d</sup> mice and 7 normal littermates, orally infected with 400 infective muscle larvae, were killed 40-42 days after infection. After the pepsin digestion of the skinned and eviscerated carcasses of the mice, the number of muscle larvae was determined under a dissection microscope at  $\times 20$  magnification.

Four SI/SI<sup>d</sup> mice and 3 normal littermates were injected intravenously with 2500 NBL suspended in 0.5 ml Hank's solution. On day 33 after injection, the number of muscle larvae was determined.

#### *Worm fecundity in vitro*

Worm fecundity was determined by the degree of NBL production of female worms *in vitro*.<sup>11)</sup> On day 6 after infection with 450 infective muscle larvae, the adult worms were obtained from the small intestine of 4 SI/SI<sup>d</sup> mice and 4 normal littermates. Sixty to one hundred worms from each mouse were placed in 10 ml of Hank's solution containing 25% v/v normal rabbit serum and incubated at 37°C for 24 hours in 5% CO<sub>2</sub> atmosphere. The number of shed NBL was determined under a microscope at  $\times 40$  magnification.

### *Histological examination*

Two-cm pices of the small intestine were fixed with Carnoy's fixative for 1 hour, embedded in paraffin wax and sectioned at 4  $\mu\text{m}$ . Sections were stained with Alcian blue-8GX (0.1% w/v in 0.7 N-HCl) and safranin O (0.5% w/v in 0.02 M acetate buffer, pH 5.0) by modification of the methods of Mayrhofer and Fisher.<sup>14)</sup> SMC and GL were counted under a magnification of  $\times 40$ . Cells in 5 random fields of a 200  $\mu\text{m} \times 200 \mu\text{m}$  square of mucosa were counted.

### *Indirect hemagglutination test (IHA)*

IHA serum titre was measured by the methods of Kamiya and Tanaka.<sup>10)</sup> Antigen was prepared by washing the infective muscle larvae in distilled water followed by lyophilization. One hundred volumes of 0.15 M phosphate buffer saline pH 7.2 were added and sonicated for 20 min at 4°C. After extracting the homogenate for 24 hours at 4°C, it was centrifuged at 16,000 G for 30 min at 4°C. The supernatant was collected and lyophilized. IHA test was conducted using 0.5% formalinized sheep tanned red blood cells.

### *Passive cutaneous anaphylaxis (PCA)*

PCA reaction was performed according to the method as described by Ovary et al.<sup>26)</sup> using Wistar rats as recipients. To prepare the larval somatic antigen, 0.15 M phosphate buffer saline pH 7.2 were added to infective muscle larvae obtained from Wistar rats, and sonicated for 20 min. The supernatant was stored at  $-80^\circ\text{C}$  and used as antigen. A 0.05 ml aliquot of pooled and diluted mouse sera was injected intradermally to recipients, and sensitized for 48 hours. Then 0.8 ml of antigen (protein concentration, 10.62 mg/ml), mixed with 0.2 ml of Evans Blue dye (2.5%), was injected intravenously at the end of sensitization period. The rats were killed after a latent period of 30 min and the diameter of reactions was measured on the internal surface of the skin. The highest serum dilution giving a 0.5 cm reaction at the skin site was considered the IgE antibody titer of the serum. These tests were duplicated.

## RESULTS

### *Kinetics of SMC in the small intestine*

The number of SMC in the small intestine of SI/SI<sup>d</sup> mice and normal littermates were enumerated during the course of the primary and tertiary infections (Figure 1). Before infection, MMC were hardly seen in the small intestine of not only SI/SI<sup>d</sup> mice but also normal littermates. In SI/SI<sup>d</sup> mice, during the course of infection in both primary and tertiary infections, no accumulation of SMC was observed. In contrast, the number of SMC increased in normal littermates in response to *T. spiralis* infection. In the primary infection the number of SMC increased slightly between days 11 and 21

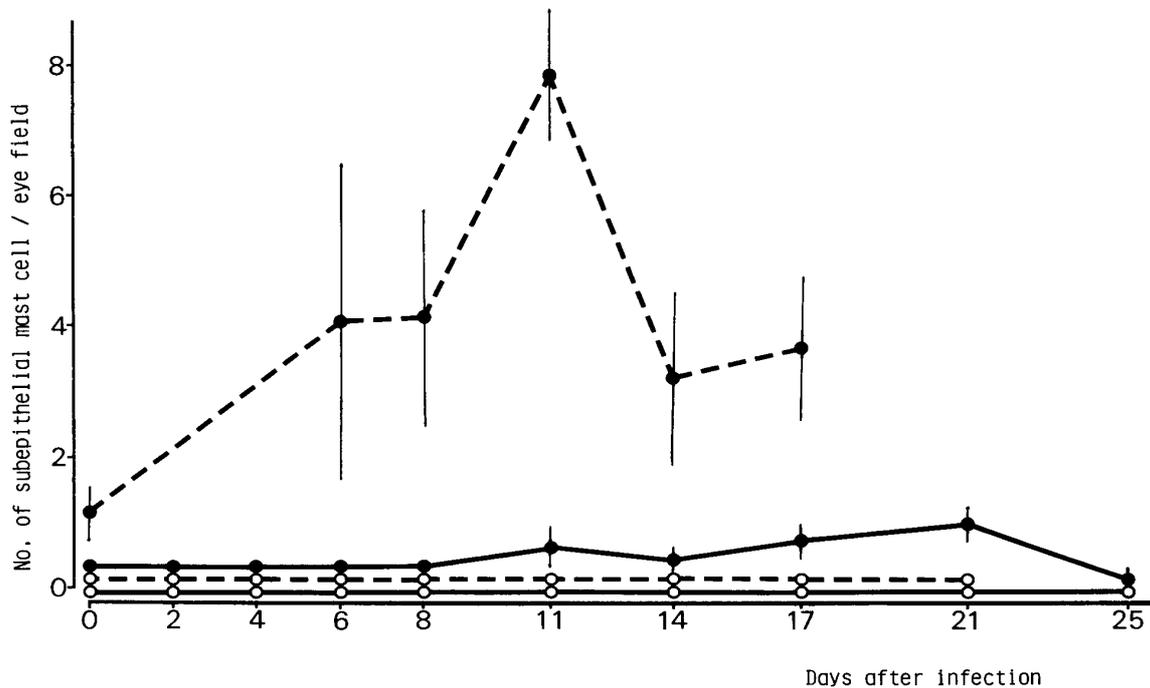


FIGURE 1 Number of subepithelial mast cells counted per eye field ( $200\mu\text{m}\times 200\mu\text{m}$ ) in primary (continuous line) and tertiary (interrupted line) infections of 3-6 SI/SI<sup>d</sup> mice (○) and 4-10 normal littermates (●). Vertical bar indicates standard deviation.

after infection, whereas in the tertiary infection a significant increase of the number of SMC was observed between days 6 and 17 after infection. The maximum number of SMC observed in tertiary infection was about 7.5 times higher than that in the primary infection.

#### *Kinetics of GL in the small intestine*

The number of GL in the small intestine of SI/SI<sup>d</sup> mice and normal littermates was enumerated during the course of primary and tertiary infections (Figure 2). Like SMC, very few or no GL was seen in the intestine of both SI/SI<sup>d</sup> mice and normal littermates before infection. In SI/SI<sup>d</sup> mice, during the course of primary and tertiary infections, very few or no GL was seen and no increase in the number of GL was observed in the small intestine. But the number of GL of normal littermates greatly increased in both primary and tertiary infections. The response of GL in the tertiary infection was faster and greater than that in the primary infection. The increase of the number of GL was significantly greater than that of the number of SMC in both of the primary and tertiary infections.

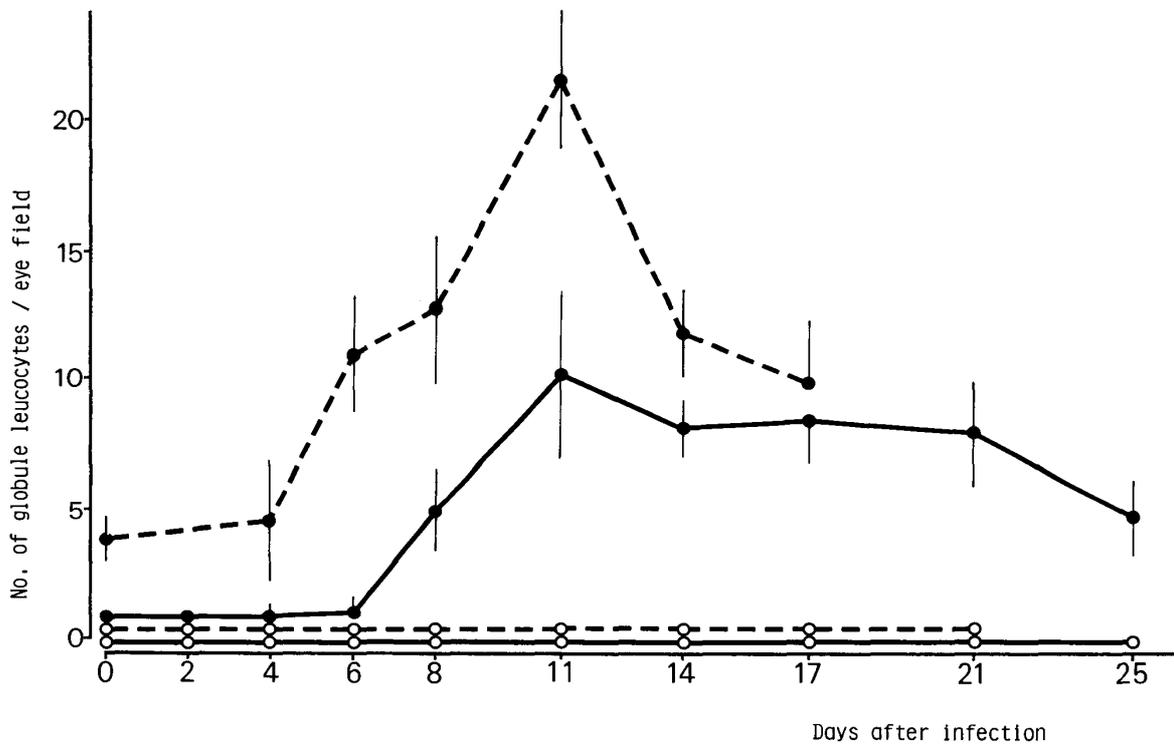


FIGURE 2 Number of globule leucocyte counted per eye field ( $200\ \mu\text{m} \times 200\ \mu\text{m}$ ) in primary (continuous line) and tertiary (interrupted line) infections of 3–6 SI/SI<sup>d</sup> mice (○) and 4–10 normal littermates (●). Vertical bar indicates standard deviation.

#### *Expulsion of T. spiralis from the intestine*

The course of the primary and tertiary infections differed in both SI/SI<sup>d</sup> mice and normal littermates (Figure 3). Worm expulsion in SI/SI<sup>d</sup> mice began later than that in normal littermates, and the worms survived for longer time in the intestine of SI/SI<sup>d</sup> mice. In contrast to the primary infection, the number of worms in normal littermates decreased dramatically in tertiary infection. On day 6 after challenge infection almost no worm was found in the intestine of normal littermates, whereas a few worms were found in the intestine of SI/SI<sup>d</sup> mice on day 21 after infection. Compared with the normal littermates, in SI/SI<sup>d</sup> mice the time course of worm expulsion following the tertiary infection did not differ much from that of the primary infection.

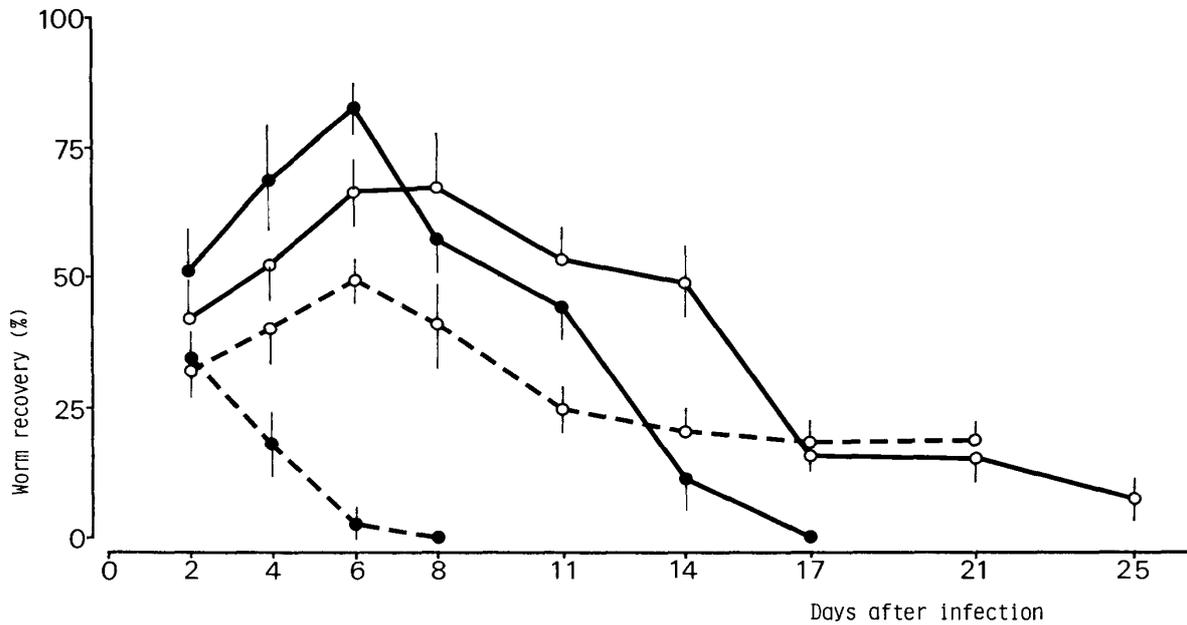


FIGURE 3 Worm burdens in small and large intestines of 3-6 SI/SI<sup>d</sup> mice (○) and 4-10 normal littermates (●) after primary (continuous line) and tertiary (interrupted line) infections. Vertical bar indicates standard deviation.

*Muscle larvae and fecundity of the adults*

In oral infection with infective larvae, the number of recovered muscle larvae in the SI/SI<sup>d</sup> mouse was about three times higher than that of the normal littermates (Table 1). However, in intravenous injection with NBL, no significant difference in the number of recovered muscle larvae was noted in SI/SI<sup>d</sup> mice and normal littermates.

TABLE 1 Larval recovery in SI/SI<sup>d</sup> mice and normal littermates inoculated with infective muscle larvae or new born larvae (NBL) of *Trichinella spiralis*\*

Mice	Inoculum		No. of recovered larvae (mean ± SD)
	Route	No. of parasite stage	
SI/SI <sup>d</sup>	per os	400 Infective larvae	35064 ± 4450 <sup>a</sup>
normal littermates	per os	400 Infective larvae	12575 ± 3823 <sup>b</sup>
SI/SI <sup>d</sup>	iv	2500 NBL	826 ± 137 <sup>c</sup>
normal littermates	iv	2500 NBL	669 ± 200 <sup>d</sup>

\* Mice were killed 40-42 days after oral inoculation or 33 days after intravenous injection.  
 P value Student's t-test a - b: P < 0.001  
 c - d: 0.2 < P < 0.5

The fecundity of adult females from SI/SI<sup>d</sup> mice was higher than that from normal littermates. The number of shed larvae/female worm, obtained on day 6 after infection, in 24 hours was  $78.4 \pm 20.7$  (SD) and  $40.5 \pm 9.0$  (values using Student's t-test:  $0.05 > P > 0.02$ ), from SI/SI<sup>d</sup> mice and +/+ mice, respectively.

#### *Serum titers of antibodies*

No significant difference in titers of antibody against *T. spiralis* in both SI/SI<sup>d</sup> mice and normal littermates was noted as shown by IHA serum titer (Figure 4) and by IgE titer measured by PCA reaction (Figure 5). But in primary infection, antibodies titer in normal littermates appeared 3–4 days earlier than that in SI/SI<sup>d</sup> mice. In tertiary infection, the titers of IHA antibodies (128–512) and IgE (640–5120) were maintained in both SI/SI<sup>d</sup> mice and normal littermates.

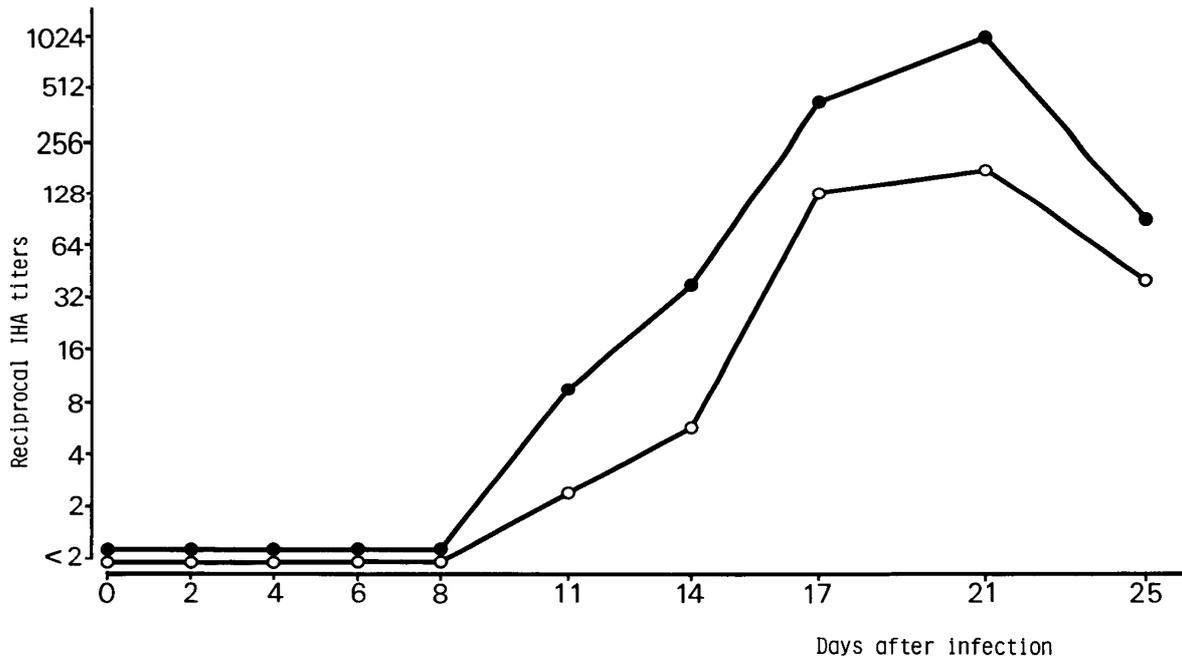


FIGURE 4 Time-course development of mean IHA titer in sera from 3–4 SI/SI<sup>d</sup> mice (○) and 4–6 normal littermates (●) infected with *Trichinella spiralis*.

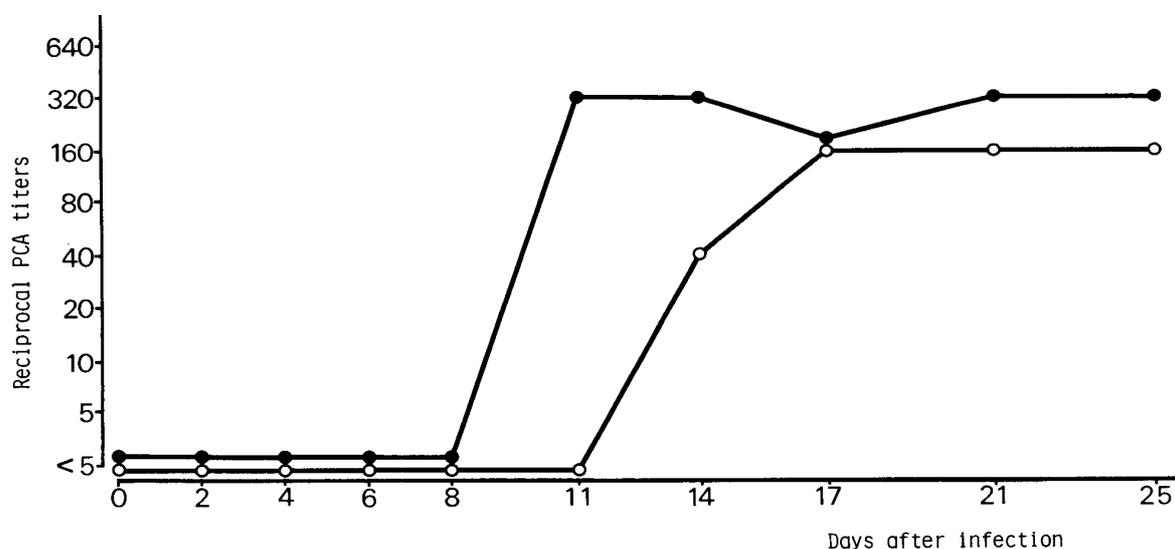


FIGURE 5 Time-course development of PCA titer in pooled sera from 3-4 SI/SI<sup>d</sup> mice (○) and 4-6 normal littermates (●) infected with *Trichinella spiralis*.

#### DISCUSSION

Connective tissue mast cell-deficiency in adult SI/SI<sup>d</sup> mice has been reported.<sup>12)</sup> In the present investigation, the SI/SI<sup>d</sup> mouse had very few or no SMC and GL before infection. Furthermore, no accumulation of SMC and GL in response to helminthic infection in the parasitized small intestine was observed. *T. spiralis* infection was prolonged in primary infection in SI/SI<sup>d</sup> mice. In the primary infection of *N. brasiliensis*, the expulsion of the worm was delayed to some extent in W/W<sup>v</sup> mice.<sup>16)</sup> And recently it was also reported that the expulsion of *T. spiralis* was delayed in W/W<sup>v</sup> mice.<sup>8,10)</sup> In W/W<sup>v</sup> mice mast cell-reconstitution accelerated expulsion of *T. spiralis* and *Strongyloides ratti*,<sup>20,23)</sup> but not that of *N. brasiliensis*.<sup>2)</sup> In secondary infection with *N. brasiliensis*, eggs were detected neither in W/W<sup>v</sup> nor in their normal littermates.<sup>3)</sup> In the present investigation, the expulsion of *T. spiralis* in SI/SI<sup>d</sup> mice was delayed not only in primary, but also in tertiary infection. The mechanism of expulsion of adult *T. spiralis* may differ from that of *N. brasiliensis*. The former parasitized in the epithelium,<sup>34)</sup> whereas the latter in the lumen.

In the present investigation, it was suggested that the higher number of recovered muscle larvae in SI/SI<sup>d</sup> mice was due to not only the long survival of the adult worms, but also their high fecundity in the intestine of SI/SI<sup>d</sup> mice.

Some hypothesis on the function of MMC with relation to the worm expulsion has been proposed. The "leak lesion" hypothesis advanced by Murray et al.<sup>17)</sup> proposed that mast cell-released amines induced mucosal permeability changes which promoted anti-worm antibody and macromolecules to be released into the lumen of parasitized intestine. Ogilvie and Jones<sup>22)</sup> suggested that the action of protective antibodies alone could not be a cause of *N. brasiliensis* expulsion and that cell-mediated damage was subsequent to the antibody-mediated damage to the worms. And the increase in the permeability of the intestinal mucosa might be caused by mechanical or toxic damages on the host which was induced by parasite.<sup>19)</sup> It is well-known that mast cells release various chemical mediators. The direct effect of 5-hydroxytryptamine on *Trichostrongylus colubriformis* has been reported.<sup>28)</sup> And also, the components of mucus from *T. colubriformis*-resistant sheep which have some properties of slow reacting substance of anaphylaxis showed the larval migration inhibitory activity.<sup>5)</sup>

Involvement of bone marrow cells in the expulsion of *T. spiralis* was proposed by some investigators.<sup>8,23,32,33)</sup> The possibility of a defect in the function of bone marrow-derived cells other than SMC and GL in the expulsion cannot be ruled out because anemia and the lack of mast cell in SI/SI<sup>d</sup> mice are due to the defect in the tissue which locally induced the differentiation of hematopoietic cells.

There is no significant difference in the serum titers of antibodies between SI/SI<sup>d</sup> and normal littermates as detected by IHA and PCA (IgE). Kojima et al.<sup>13)</sup> reported that the carrier effect on antihapten IgE antibody response was demonstrated in W/W<sup>v</sup> mice in which showed prolonged infection of *N. brasiliensis*. But we cannot conclude that IgE production in SI/SI<sup>d</sup> mice is normal because SI/SI<sup>d</sup> mice have only very few mast cells and showed somewhat delayed response of IgE to *T. spiralis*. Moreover, we determined only the serum titer of IgE which binds locally with very high affinity to mast cells. It has been reported a protective aspect of IgE against the larvae in muscles. Strains of IgE high responder mice to *T. spiralis* infection showed fewer muscle larvae than that of low responder mice, whereas no correlation between worms in the intestine and IgE titers in the high and low responder mice could be demonstrated.<sup>27)</sup> Dessein et al.<sup>4)</sup> reported that rats, whose IgE were depleted by injection with anti  $\epsilon$ -chain antibodies, harbored more larvae in their muscles than in the control. *Trichinella* infection induces a profound tissue and blood eosinophilia. Eosinophils have been shown to act as efficient anti-helminth effector cells.<sup>6)</sup> Mast cells contain and release various eosinophil chemotactic factor.<sup>21)</sup> However, eosinophilia in small intestine and muscles was observed in SI/SI<sup>d</sup> mice.<sup>24)</sup>

Although various factors which involved in the expulsion of worms from the intestine and which damage worms have been reported, our results suggest that SMC and GL might be involved in the resistance to the intestinal phase of *T. spiralis*.

## ACKNOWLEDGEMENTS

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