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## CROSS RESISTANCE AGAINST CHALLENGE INFECTION IN MICE INFECTED WITH *TRICHINELLA SPIRALIS* OR *T. PSEUDOSPIRALIS*

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Worm recovery from the small intestine was carried out in ICR mice infected with either *Trichinella spiralis* or *T. pseudospiralis* and then homologously or heterologously challenged 14 or 42 days after the primary infection. Significantly less worms were recovered from challenge infections as compared to the naive positive controls. Less worms were expelled from mice challenged on day 42 than on day 14 after the primary infection. Significantly more worms were retained in the groups of mice primarily infected with *T. spiralis* and then challenged with *T. pseudospiralis* as compared to the other three challenged groups. This was observed in both the groups of mice challenged on days 14 and 42 after the primary infection. A correlation between the number of worms retained in each mouse and the number of intestinal mucosal mast cells in the individual mouse was observed. No significant difference was observed in the number of muscle larvae recovered by digestion from among the challenge and the control groups of infected mice. However, in histopathological examination of *T. spiralis* infected mice challenged with *T. pseudospiralis* on day 42 after primary infection, almost no *T. pseudospiralis* larvae were observed in the muscle but when the infection protocol was reversed, the muscle section showed about 40% *T. spiralis* larvae and 60% *T. pseudospiralis* larvae.

Key words: *Trichinella spiralis*, *T. pseudospiralis*, mast cell, cross resistance.

### INTRODUCTION

It is widely known that resistance to intestinal parasites can take the form of intestinal expulsion from the host which involves immunological and non-immunological mechanisms. These resistances can be elicited by an existing species of parasite in the host against subsequent infection by a different species of parasite. This form of cross resistance has been reported to occur against *Trichinella spiralis* in rodents

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following infection with *Nippostrongylus muris* (= *N. brasiliensis*) (LOUCH, 1962), *Trichuris muris* (BRUCE & WAKELIN, 1977), *Angiostrongylus cantonensis* (AU & KO, 1979), *Strongyloides ratti* (MOQBEL & WAKELIN, 1979) and *Eimeria nieschulzi* (STEWART et al., 1980). Resistance induced in the host by *T. spiralis* against the *N. brasiliensis* (KAZACOS, 1975), *T. muris* (BRUCE & WAKELIN, 1977) and *Hymenolepis diminuta* (SILVER et al., 1980) had also been reported.

The cross resistance between different species of intestinal parasites may be brought about the presence of common antigen on them and thus specific humoral and cell-mediated responses may be involved. However, it has been suggested that the final effector mechanism may be non-specifically acting inflammation of the intestine (LARSH & RACE, 1975). Rejection of parasites from the gut has also been attributed to the residual inflammation of the host which has been elicited by a preceding infection with a parasite bearing no functional common antigen with the following parasite and which itself has already been expelled.

The involvement of the intestinal mucosal mast cell in the expulsion of *T. spiralis* has been implicated by the delayed expulsion and the absence of the intestinal mast cells in nude mice (RUITENBERG & ELGERSMA, 1976), nude rats (VOS et al., 1983) and mast cell deficient  $W/W^V$  mice (KAMIYA et al., 1983; HA et al., 1983; OKU et al., 1984; ALIZADEH & MURRELL, 1984).

Expulsion of *T. spiralis* from the gut in homologous challenge had been reported (WAKELIN & LLOYD, 1976; FAUBERT, 1977). However, details of heterologous challenge involving *T. pseudospiralis* need further studies.

Unlike *T. spiralis*, *T. pseudospiralis*, which was first isolated from a raccoon in north Caucasus by GARKAVI in 1972, has comparatively smaller and non-encapsulating larvae in the muscle (GARKAVI, 1972). The newborn larvae of *T. pseudospiralis* are also known to be able to develop to maturity in the muscle of the avian host (TOMASOVICOVA, 1975), whereas this is not so in the case of *T. spiralis* (OOI et al., 1984). The absence of a capsule around the muscle larvae of *T. pseudospiralis* in the mammalian host and its ability to successfully infect the avian host suggest a parasite-host relationship which is different from that of *T. spiralis* (ALKARMI & FAUBERT, 1981).

We report herein, the cross resistance against challenge infection on days 14 and 42 after the primary infection with *T. spiralis* or *T. pseudospiralis* in ICR mice. Correlation between the number of intestinal mucosal mast cells and the number of worms remaining in the gut was also examined.

#### MATERIALS AND METHODS

*T. spiralis* used was isolated from a pig in Poland and kindly provided by Prof. YAMAGUCHI of Hirosaki University. *T. pseudospiralis* used was isolated from a raccoon by Garkavi, and kindly provided by Prof. WONG of University of California, Davis.

The experimental animals used were ten-week-old male ICR mice purchased from

Nihon Clea, Japan. Infective larvae of *T. spiralis* or *T. pseudospiralis* were obtained by digesting the carcasses of the infected mice with 0.5% pepsin-HCl for 4 hr at 37°C. After washing the larvae repeatedly by simple sedimentation in physiological saline solution, 200 of these larvae were orally inoculated into groups of five mice. Challenge infection with 200 larvae of either *T. spiralis* or *T. pseudospiralis* were given orally to the mice either at 14 or 42 days after the primary infection. For the investigation of the intestinal phase, the mice were killed 5 days after the challenge infection and the total number of adult worm in the intestine was recovered. For the investigation of the muscle phase, the mice were killed 60 days after the challenge infection and the total number of muscle larvae in the individual mice were determined after pepsin digestion of the carcass. At the time of challenge infections, groups of five non-infected mice were orally given 200 larvae of *T. spiralis* or *T. pseudospiralis* as control, in order to confirm the viability and infectivity of the challenge infection. The infection protocol of the mice for investigating the intestinal phase and the muscular phase are shown in Tables 1 and 2, respectively.

After the mice were killed, their small intestines were removed and divided into four portions of equal length. About 1 cm of the anterior tip of the portions was fixed in Carnoy's fixative for histological sections. The rest of the small intestine was slit longitudinally and the mucosal layer scraped with forceps. All the worms in the mucosal scrapings were counted under the dissection microscope. The remaining sub-mucosal and muscular layers of the small intestine were placed on wire mesh in a petri dish containing a warm saline solution and then incubated at 37°C for 4 hr to recover the remaining worms.

Histological sections of the small intestine were stained with astra blue and counterstained with acid fuchsin. Mast cells in the lamina propria had been referred to as subepithelial mast cells and those in the epithelia as globule leucocytes (RUITENBERG et al., 1979). Since it is still controversial whether these mast cells are two independent cell populations or not (GREGORY, 1979), here, they are referred to collectively as mucosal mast cell and were enumerated by randomly counting them at X200 magnification covering an area of 0.5 mm<sup>2</sup> per mouse.

In order to determine the ratio of *T. spiralis* to *T. pseudospiralis* larvae in the muscle of mice given heterologous challenge infection, groups of 5 mice which were infected orally with 200 larvae in primary infection, were heterologously challenged 42 days later with 200 larvae and killed 60 days after the challenge infection. The diaphragms of these mice were fixed in Carnoy's fixative and the histological sections prepared were stained with haematoxylin-eosin. Muscle larvae surrounded by a cyst wall and confined in a "contracted" infected muscle cell were identified as that of *T. spiralis* and those without a cyst wall and the infected muscle cell remained "elongated" were identified as *T. pseudospiralis*. The number of these two types of larvae were counted in the individual mouse and their ratio determined. The ratio of the

two types of larvae was determined by the average ratio of the 5 mice in each group.

The Student's t-distribution test was used to analyse the number of larvae recovered. The data were considered statistically significant if the "t" value was above the five percent level.

#### RESULTS

The recovery rate of the adult worms from the small intestine, of groups of five mice 5 days after the challenge infection with 200 *T. spiralis* or *T. pseudospiralis* larvae, is shown in Table 1. Significantly less worms were recovered from the challenge infection given on days 14 and 42 after the primary infection as compared with the positive controls. Less worms were expelled from mice challenged on day 42 than on day 14 after the primary infection. More worms were retained significantly in the groups of mice primarily infected with *T. spiralis* and then challenged with *T. pseudospiralis* as compared with the other three challenged groups. This phenomenon was observed in both the groups of mice challenged on days 14 and 42 after the primary infection.

The correlation between the number of worms recovered from each mouse and

TABLE 1 Recovery of adult worms from the small intestine of groups of five mice 5 days after challenge infections with 200 *Trichinella spiralis* or *T. pseudospiralis* larvae.

Primary infection Inoculum	Challenge infection		Mean no. $\pm$ S. D. of worms
	Days	Inoculum	
<i>T. spiralis</i>	14	<i>T. pseudospiralis</i>	10.2 $\pm$ 2.78
"	42	"	105.0 $\pm$ 40.75
<i>T. spiralis</i>	14	<i>T. spiralis</i>	0.2 $\pm$ 0.45
"	42	"	64.8 $\pm$ 34.90
<i>T. pseudospiralis</i>	14	<i>T. pseudospiralis</i>	1.8 $\pm$ 3.49
"	42	"	30.0 $\pm$ 46.67
<i>T. pseudospiralis</i>	14	<i>T. spiralis</i>	0.6 $\pm$ 1.34
"	42	"	52.8 $\pm$ 18.70
Uninfected	14	<i>T. pseudospiralis</i>	142.0 $\pm$ 8.20
"	42	"	149.8 $\pm$ 15.74
Uninfected	14	<i>T. spiralis</i>	128.2 $\pm$ 24.00
"	42	"	134.2 $\pm$ 15.07



observed. That is, mice harbouring more worms have lower intestinal mast cells count. This is much evidenced in mice challenged on day 42 after the primary infection.

The recovery rate of the muscle larvae from the infected control and challenged mice is shown in Table 2. No significant difference was observed among the challenge and the control groups of mice. In *T. spiralis* infected mice challenged with *T. pseudospiralis* 42 days after the primary infection and killed 60 days later, the ratio of *T. spiralis* to *T. pseudospiralis* larvae in the diaphragm muscle section, after counting a total of 213 larvae, was 99.7% to 0.3%. However, when the infection protocol was reversed, the muscle section showed 39.8% *T. spiralis* larvae and 60.2% *T. pseudospiralis* larvae after counting a total of 160 larvae. Figure 2 shows the establishment of *T. spiralis* larvae in the muscle of a mouse primarily infected with *T. pseudospiralis*

TABLE 2 Recovery of muscle larvae from groups of four or five mice 60 days after after challenge infections with 200 *Trichinella spiralis* or *T. pseudospiralis* larvae.

Primary infection Inoculum	Challenge infection		Mean no. of larvae $\pm$ S. D.
	Days	Inoculum	
<i>T. spiralis</i>	14	<i>T. pseudospiralis</i>	30,570 $\pm$ 4,830
"	32	"	31,180 $\pm$ 10,640
<i>T. spiralis</i>	14	<i>T. spiralis</i>	32,670 $\pm$ 5,710
"	42	"	38,080 $\pm$ 9,600
<i>T. pseudospiralis</i>	14	<i>T. pseudospiralis</i>	27,280 $\pm$ 3,440
"	42	"	21,940 $\pm$ 4,910
<i>T. pseudospiralis</i>	14	<i>T. spiralis</i>	24,240 $\pm$ 9,870
"	42	"	23,380 $\pm$ 6,900
<i>T. pseudospiralis</i>	14	Uninfected	18,900 $\pm$ 10,700
"	42	"	23,680 $\pm$ 5,730
<i>T. spiralis</i>	14	Uninfected	25,610 $\pm$ 6,560
"	42	"	29,000 $\pm$ 5,730
Uninfected	14	<i>T. pseudospiralis</i>	23,560 $\pm$ 4,430
"	42	"	19,900 $\pm$ 2,340
Uninfected	14	<i>T. spiralis</i>	31,730 $\pm$ 11,099
"	42	"	27,450 $\pm$ 7,140

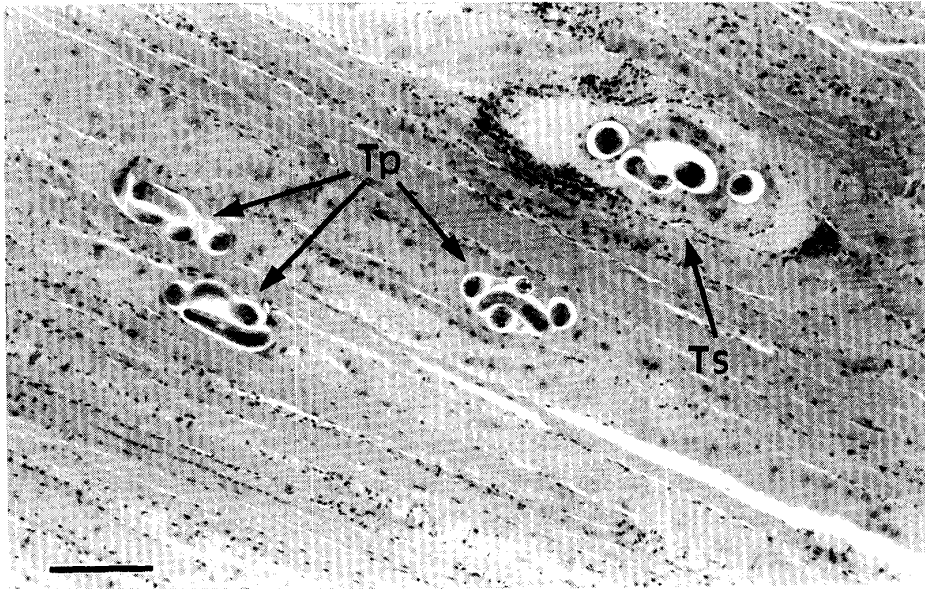


FIGURE 2 Diaphragm muscle section of a mouse primarily infected with *T. pseudospiralis*(Tp), challenged 42 days later with *T. spiralis* (Ts) and then killed 60 days after the challenge infection. Tp and Ts are seen next to each other. Haematoxylin-eosin. Bar=0.1 mm.

and then challenged with *T. spiralis*.

#### DISCUSSION

Expulsion of *T. spiralis* from the small intestine has been attributed first to the cell-mediated immunologically specific hypersensitivity reaction which then leads to a non-specific inflammation of the intestinal wall (LARSH & RACE, 1975). In the process of the expulsion, antibody-mediated damage of the worms has also been suggested (LOVE et al., 1976). Besides this spontaneous expulsion of the worms, a phenomenon of rapid expulsion is seen in previously immunized rat and certain strain of mice in which worms in the challenge infection were expelled within a day or two.

In our experiment, ICR mice, an outbred strain, showed rejection of not only *T. spiralis* but also *T. pseudospiralis* challenged on day 14 after the primary infection. Significantly more worms were expelled from the mice in all challenge groups on day 14 rather than on day 42 after the primary infection. The rejection of these worms may be due to the mechanism of rapid expulsion and/or spontaneous expulsion, which are both strongly evident when challenged on day 14 after the primary infection. In contrast, only spontaneous expulsion may be involved in the rejection of the intestinal worms when challenged on day 42 after the primary infection. This is reflected by the subsiding of the inflammatory elements in the gut during this time. The results of



PALMAS et al. (1985) on the stimulation of immunity by *T. spiralis* and *T. pseudospiralis* infections to homologous and heterologous challenge infection carried out on day 21 after primary infection were similar to that of our day 14 challenge infection.

Since *T. spiralis* and *T. pseudospiralis* are sibling species, both of them were shown to have common as well as distinct antigens (EFREMOV & ERMOLIN, 1981; ALKARMI & FAUBERT, 1985). Thus, rejection of either parasites in the challenge infection may not only be due to the residual effect of inflammation elicited by the preceding infection but also may be due to the specific cross immunity attributed to the common functional antigens. Therefore, it is suggested that the mechanism which expels *T. pseudospiralis* is also the same as that which expels *T. spiralis*. However, this needs further investigation. The significantly greater number of *T. pseudospiralis* worms recovered from the intestine of *T. spiralis* infected mice which had been challenged later with *T. pseudospiralis* suggest that a greater number of *T. pseudospiralis* was able to evade the host immune response elicited by *T. spiralis*. Nevertheless, the elucidation of this phenomenon awaits further studies.

When the infected mice were challenged on day 42 after the primary infection, we observed that the number of worms retained in the small intestine of the individual mice showed a good correlation with the number of intestinal mast cells. It is thus tempting to suggest that the kinetics of the mast cell is inversely proportional to the number of worms retained in the gut. The role of the intestinal mucosal mast cells in the expulsion of *T. spiralis* has been reviewed by LEE et al. (1986).

Cross immunity between *T. pseudospiralis* in rats and mice at the muscle phase had been reported (GARKAVI, 1974; BRITOV, 1975). However, our results showed that the recovery of muscle larvae by digestion of the challenged mice and comparing their number with the infected control is not a good indicator of the resistance to secondary infection. This is because the number of muscle larvae recovered from mice which were first infected with *T. pseudospiralis* and then challenged with *T. spiralis* 42 days later, were not significantly different from the number of larvae recovered from mice which were infected with only *T. pseudospiralis*. This gives the impression that immunity to the challenge *T. spiralis* infection has been manifested. However, histopathological examination of the muscle of the challenged mice showed that *T. spiralis* do establish infection in primarily *T. pseudospiralis* infected mice. Thus it is suggested that, histological examination of the muscle of the challenged mice may provide a clearer picture of the degree of cross immunity at the muscle phase.

Immune modulation of the host systemic response by the muscle phase of *T. pseudospiralis*, as manifested by the suppression of myositis, has been reported (GABRYEL et al., 1981; STEWART et al., 1985). In *T. spiralis* infection, myositis is spectacular. Myositis may account for the absence the muscle larvae of *T. pseudospiralis* in *T. spiralis* infected mice given heterologous challenge infection. In *T. spiralis* infected mice challenged with *T. pseudospiralis*, inflammatory cells in the muscle may

elicit an environment which is non-habitable for the newborn *T. pseudospiralis* larvae. However, when the primary infection was carried out with *T. pseudospiralis*, it is suggested that not enough inflammatory cells were present to prevent the newborn larvae of *T. spiralis* from establishing infection. This may account for the mixed infection of *T. spiralis* and *T. pseudospiralis* larvae. A substantial decline in the number of *T. pseudospiralis* muscle larvae in mice concurrently infected with *T. spiralis* had been reported (PRZYJALKOWSKI et al., 1981; STEWART et al., 1985). This concurred well with our observation that the inflammation caused by the presence of *T. spiralis* in the muscle is detrimental to the survival of *T. pseudospiralis*.

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