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The effect of concurrent infection with *Trichinella spiralis* on *Hymenolepis microstoma* in mice

R. J. HOWARD, P. R. CHRISTIE,* D. WAKELIN, M. M. WILSON *and* J. M. BEHNKE†

Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Bearsden Road, Bearsden, Glasgow, G61 1QH

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SUMMARY

The intestinal changes brought about by rejection of *Trichinella spiralis* from mice were studied in relation to their effects on a concurrent infection with *Hymenolepis microstoma*, a cestode not normally rejected from mice. The rejection phase of *T. spiralis* was associated with a marked stunting of growth of *H. microstoma* given just before, during, or just after rejection of the nematode. The survival of *H. microstoma* was affected only when rejection of *T. spiralis* coincided with the intestinal phase of the cestode: if *T. spiralis* rejection was timed to occur after the scolex of the cestode had entered the bile duct there was no loss of *H. microstoma*. It is suggested that the adverse effects on growth and establishment of *H. microstoma* were due to the non-specific inflammatory component of the host's response to infection with *T. spiralis*.

INTRODUCTION

The expulsion of *Nippostrongylus brasiliensis* from rats has been reported to require not only an antibody response and a specific cellular element but also a non-specific element derived from bone marrow (Dineen & Kelly, 1973). After reviewing the immune mechanisms of various nematode infections, Ogilvie (1974) suggested that 'whatever the exact sequence of immunological events which trigger expulsion (of nematodes) it is probably caused by the release of some non-specific effector'.

Bruce & Wakelin (1977) showed that the expulsion of *Trichinella spiralis* from mice resulted in the non-specific expulsion of a concurrent *Trichuris muris* infection and that this effect was reduced by the administration of the anti-inflammatory drug indomethacin. A similar non-specific rejection of *Hymenolepis diminuta* from mice is associated with the rejection of a concurrent *T. spiralis* infection and it was suggested that this was attributable to the inflammation in the intestine initiated by the latter parasite (Behnke, Bland & Wakelin, 1977). These observations on interspecific interactions were extended in the present

* Reprint requests to P. R. Christie.

† Present address: Department of Zoology, University of Nottingham, University Park Nottingham, NG7 2RD.

report by determining the effect of a *T. spiralis* infection upon a concurrent *H. microstoma* infection. In contrast to all the above parasites, *H. microstoma* is not normally rejected from mice (Moss, 1971; Howard, 1977), although a secondary infection grows more slowly than a primary infection (Tan & Jones, 1967, 1968; Howard, 1976). The work presented in this paper was initiated to establish whether or not *H. microstoma* is susceptible to rejection processes, utilizing the severe host response to *T. spiralis* infection.

MATERIALS AND METHODS

Helminth-free male and female NIH (Anglia Laboratory Animals) mice were used in Experiments 1 and 2 respectively, male C3H (Bantin and Kingman Limited, Yorkshire) mice were used in Experiment 3, and outbred male CFLP (bred at the Wellcome Laboratories) mice were used in Experiment 4. All mice received food and water *ad libitum*.

The infection and autopsy procedures have been described previously for *H. microstoma* (Howard, 1976, 1977) and *T. spiralis* (Wakelin & Lloyd, 1976*a*). Briefly, mice received cysticercoids of the cestode by stomach tube whilst under light ether anaesthesia. The cestodes were recovered by slitting open the bile duct and small intestine and incubating these tissues at 37 °C for 1–2 h. Infective larvae of *T. spiralis* were recovered from rats or mice by digestion of the animals in acid pepsin for 2 h at 37 °C. These larvae were suspended in 0·2 % agar and the mice were infected with 500 (Exps 1 and 3) or 350 (Exps 2 and 4) larvae by oral inoculation into the stomach with a syringe and blunted cannula. The adult nematodes were recovered by a modified Baermann technique.

The dry weights and numbers of cestodes recorded from different groups of mice were compared using the Wilcoxon rank sum test (Remington & Schork, 1970).

NIH mice reject *T. spiralis* starting on day 8 of infection and rejection is complete by day 11·5 (Wakelin & Lloyd, 1976*a*); C3H and CFLP mice are slower in rejecting *T. spiralis*, but major worm loss generally occurs between days 10 and 12, rejection being complete by day 18 (Wakelin, unpublished observations).

RESULTS

The effect of a T. spiralis *infection on the growth and survival of a pre-existing* H. microstoma *infection*

Investigation of the effect of *T. spiralis* infection on a pre-existing *H. microstoma* infection began with experiments (Exps 1 and 2) in which groups of mice were infected with either 5 cysticercoids of *H. microstoma*, or *H. microstoma* and *T. spiralis* 5 days later, or *T. spiralis* only. These groups were infected and killed as shown in Table 1, and the results are given in Table 2.

The establishment and rejection of the nematode were apparently not affected by concurrent infection with the cestode (Group B). Similarly, the survival of the cestode was not affected by the nematode (Groups B, E, H and K). However,

Table 1. *The effect of* Trichinella spiralis *infection on a pre-established* Hymenolepis microstoma *infection: experimental design*

(Letters in parentheses are group designations.)

Group*	Day			
Experiment 1	0	5	9	16
Hm only	Hm	—	K† (A)	K (D)
Hm + Tsp	Hm	Tsp	K (B)	K (E)
Tsp only	—	Tsp	K (C)	K (F)

	Day				
Experiment 2	0	5	13	17	35
Hm only	Hm	—	—	K (G)	K (J)
Hm + Tsp	Hm	Tsp	—	K (H)	K (K)
Tsp only	—	Tsp	K (L)	K (I)	—

* Hm, infected with 5 cysticercoids of *H. microstoma*; Tsp, infected with 500 (Exp. 1) or 350 (Exp. 2) larvae of *T. spiralis*.
† K, day of autopsy.

Table 2. *The effect of* Trichinella spiralis *infection on a pre-established* Hymenolepis microstoma *infection: results*

(Experimental mice received 5 cysticercoids of *H. microstoma* on day 0 followed by *T. spiralis* infection on day 5.)

Group*	Day of autopsy	Mean Tsp recovery	Mean Hm recovery	Mean dry weight of Hm/mouse (mg)	No. of mice
Experiment 1					
A. Hm only	9	—	5·0	6·95	10
B. Hm + Tsp	9	—	4·1	5·34	10
	9	196·8	—	—	5
C. Tsp only	9	170·8	—	—	5
D. Hm only	16	—	4·6	95·18	9
E. Hm + Tsp	16	—	4·7	59·75†	9
	16	0·4	—	—	5
F. Tsp only	16	2·2	—	—	5
Experiment 2					
G. Hm only	17	—	4·9	79·87	7
H. Hm + Tsp	17	—	4·9	55·81†	7
I. Tsp only	17	0·0	—	—	5
J. Hm only	35	—	5·0	94·64	7
K. Hm + Tsp	35	—	4·9	81·99	7
L. Tsp only	13	163·2	—	—	5

* Hm, *H. microstoma*; Tsp, *T. spiralis*.
† Significant difference ($P < 0·01$).

H. microstoma recovered from dual-infected mice were lighter than those from control mice, but the difference was significant only in mice killed immediately after rejection of the nematode had commenced (Groups E and H killed 11 and 12 days after infection with *T. spiralis* respectively). The weight of worms recovered from the dual-infected mice 30 days after *T. spiralis* infection (Group K) was lower than that from control mice but not significantly so, indicating that the stunting of the cestode was not permanent.

The establishment and growth of H. microstoma *given before, during, and after the rejection phase of* T. spiralis

The results of the previous experiments showed that the growth, but not the survival, of *H. microstoma* was affected during the expulsion of *T. spiralis*. The cestode may not have been lost during the rejection phase of *T. spiralis* because (1) the scolex of *H. microstoma* is completely resistant to the changes in the gut associated with rejection of the nematode, or (2) the scolex was protected from contact with these intestinal changes by being inside the bile duct.

In an attempt to clarify this situation, an experiment (Exp. 3) was designed in which 4 groups of *T. spiralis*-infected mice were challenged with *H. microstoma* at various times so that (1) the scolex of the cestode was inside the bile duct (3–4 days after infection) before expulsion of the nematode had begun (Group B), (2) the cestode was migrating in the intestine during the early part of the expulsion phase of the nematode (Group D), (3) as (2), but during the latter part of the expulsion phase (Group F) and (4) the cestode was establishing after the nematode had been expelled (Group H).

The scope of this experiment was extended in Exp. 4 by infecting a group of mice with *H. microstoma* before infecting with *T. spiralis*, so that the cestode had only experienced the pre-rejection phase of *T. spiralis* at autopsy (Group J); and by infecting another group as for (4) above, but later, after expulsion of the nematode. All mice were autopsied 10 days after infection with *H. microstoma* and the design and results of Exps 3 and 4 are summarized in Table 3.

In mice infected with *H. microstoma* 3 days before giving *T. spiralis* (Groups I and J) there was a similar number of cestodes recovered from dual-infected and control mice and there was no loss of weight in the cestodes recovered from the dual-infected group: in fact, the average weight was slightly higher than that from the control group.

In mice infected with *H. microstoma* 1 day after infection with *T. spiralis* (Groups A and B) there was a similar number of cestodes recovered from both groups, but the weight of the cestodes from the dual-infected group was significantly lower than that from control mice.

In mice infected with *H. microstoma* 8 or 12 days after *T. spiralis* (Groups C–D, E–F and K–L) significantly fewer cestodes were recovered from the dual-infected mice than from control mice and these worms were significantly lighter than those from control mice (the differences between Groups F and L may be attributable to the use of different strains of mice and/or different numbers of *T. spiralis* administered).

Table 3. *The effect of varying the time of* Hymenolepis microstoma *infection with respect to* Trichinella spiralis *infection*

(*T. spiralis* (Tsp) given on day 0; mice killed 10 days after *H. microstoma* (Hm) infection).

Group	Day infected with Hm	Mean Hm recovery	Mean dry weight of Hm/mouse (mg)	No. of mice
Experiment 3				
A. Hm	1	4·0	6·61	7
B. Hm+Tsp	1	3·9	1·63*	7
C. Hm	8	4·1	5·38	8
D. Hm+Tsp	8	0·6*	0·01*	8
E. Hm	12	4·5	6·78	8
F. Hm+Tsp	12	0·8*	0·11*	8
G. Hm	21	4·4	5·93	7
H. Hm+Tsp	21	3·4	1·56†	7
Experiment 4				
I. Hm	−3	4·1	6·57	9
J. Hm+Tsp	−3	4·5	7·57	8
K. Hm	+12	4·7	6·39	9
L. Hm+Tsp	+12	2·6*	1·20*	8
M. Hm	+29	4·7	4·03	9
N. Hm+Tsp	+29	4·4	3·82	9

* Significant difference ($P < 0{\cdot}01$).
† Significant difference ($0{\cdot}02 > P > 0{\cdot}01$).

In mice infected with *H. microstoma* 21 or 29 days after giving *T. spiralis* (Groups G–H and M–N), the recoveries from the dual-infected groups were not significantly lower than from controls. Cestodes recovered from the group given *T. spiralis* 21 days before infection with *H. microstoma* were significantly lighter than controls, but no significant difference in weight was evident in the group given *H. microstoma* 29 days after infection with *T. spiralis*.

DISCUSSION

The results of the experiments presented in this paper suggest that the effect of concurrent infection with *T. spiralis* on survival and growth of *H. microstoma* depends greatly on the relative timing of the infections. In mice infected with *H. microstoma* before *T. spiralis* and autopsied before rejection of the nematode had commenced, there was no significant effect on the weight of the cestode, indicating that the pre-rejection phase of *T. spiralis* had no harmful effects on the cestode.

However, *H. microstoma* which had been exposed to the rejection phase of *T. spiralis* were always lighter than those from control mice, and this deleterious effect on the cestode appeared to persist until at least 21 days after infection with the nematode. There was no significant difference between the weights of *H. microstoma* recovered from dual-infected mice and those from control mice when

infection with the cestode took place 29 days after infection with *T. spiralis*, indicating that this effect is unlikely to be due to an immunologically specific cross-reaction. Stunting of *H. microstoma* exposed to the rejection phase of *T. spiralis* was severe, but apparently not permanent: there was no significant difference between the weights of worms recovered from mice infected with *H. microstoma* only and those infected with *H. microstoma* 30 days after infection with *T. spiralis* (Table 2, Exp. 2, Groups J and K).

Rejection of *T. spiralis* from the mouse appears to involve both specific humoral and cell-mediated responses (Love, Ogilvie & McLaren, 1976; Wakelin & Lloyd, 1976*b*), but the final effector mechanism may well be a non-specifically acting inflammation of the intestine (Larsh, 1967; Larsh & Race, 1975). As there appears to be no direct cross-immunity between *H. microstoma* and *T. spiralis*, it is apparently the non-specific inflammatory component that is responsible for the observed effects on survival and growth of *H. microstoma*. However, mice reduce their food consumption during rejection of *T. spiralis* (Larsh, Goulson & Van Zandt, 1962; Goulson & Larsh, 1964) and it is well known that reduced intake of carbohydrates may retard the growth of hymenolepids (Read & Rothman, 1957*a*). The reduction in growth of *H. microstoma* may, therefore, have been at least partially due to the altered dietary intake of the mouse, although it is also conceivable that a reduced intake might be offset by the malabsorption of nutrients associated with *T. spiralis* infection (Castro, Olson & Baker, 1967). However, it is unlikely that altered intake would markedly affect the initial establishment of the worms (Read & Rothman, 1957*b*): the significant reduction in numbers of *H. microstoma* recovered that did occur in some groups was probably, therefore, caused by the inflammation associated with the rejection of *T. spiralis*. The role of inflammation in retarding growth of the cestode is less clear.

In secondary infections of *H. microstoma* in mice, worm growth is retarded by the host response during the first 4 days of infection: after entering the bile duct, growth resumes at a similar rate to that in primary infections (Howard, 1977). This resembles the situation described in the present paper in which, following establishment of the scolex of *H. microstoma* in the bile duct, the worm was not sufficiently affected by inflammation for loss to occur: it may be that changes in the intestine during inflammation do not extend into the bile duct. It is also possible that the scolex itself changes after entry into the bile duct and becomes resistant to inflammatory products; the inflammation of the peribiliary tissues which is initiated by the cestode itself does not appear to affect its survival (Lumsden & Karin, 1970), but the components in this inflammation may differ considerably from *T. spiralis*-induced intestinal inflammatory products.

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