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Comparison of the Kinetics of Infection with *Hymenolepis nana* between BALB/c and dd Strains of Mice

AKIRA ITO

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Introduction

The fecundity and longevity of the dwarf tapeworm, *Hymenolepis nana* has been found to be much lower and shorter in BALB/c mice than in dd mice (Isaak *et al.*, 1977; Ito, 1980, 1981). Such difference in fecundity and longevity of *H. nana* has been proven to be affected at least by the difference in the onset times of the acquired immune responses directed exclusively against the lumen phase of *H. nana* (Isaak *et al.*, 1977; Ito, 1980, 1981). The present study was intended to observe the dynamic feature of the primary infection of *H. nana* in these strains of mice by quantitative methods. Whether such difference described above found between the two strains of mice is not only due to the difference in the onset times of the acquired immune responses, but also due to the difference in the host's susceptibility, or innate immunity (Read, 1958; Sprent, 1969) to this parasite is discussed. It is stressed that the different figures of *H. nana* infection in the two strains of mice is primarily attributable to the difference in rapidity of the onsets of the host's immune responses directed exclusively against the lumen phase (the late response directed against cysticercoid challenges and the worm expulsion response against the established worms themselves), but not against the tissue phase (the early response directed exclusively against the egg challenges) and not attributable to the difference in susceptibility or innate immunity to this parasite infection.

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Department of Parasitology, Gifu University School of Medicine, Tsukasa-machi 40, Gifu, Japan.

Materials and Methods

Parasites

Preparation and inoculation of shell-free eggs (Berntzen and Voge, 1965) and mouse-derived cysticercoids (Ito, 1977) of *Hymenolepis nana* has been described previously (Ito *et al.*, 1978). Cysticercoids included not only encysted but also excysted ones through the preparation. Both types of the cysticercoids had similar infectivity. Viability of cysticercoids was determined by observing if the worms were stained with methylen blue or not. Dead or dying worms (scoleces) were stained showing bubbles around their body surface, but live worms either encysted or excysted were not stained.

Host animals

The basic method for the maintenance of the two (dd and BALB/c) strains of worm-free mice has been described previously (Ito, 1980). All the animals were housed under 24±2 C, 55±10% humidity condition. Food (CA-1, CLEA JAPAN,

Inc., Tokyo) and water were available *ad lib*. Throughout the experiments, five- to 6-week-old mice of both sexes were used and given the *H. nana* infection at this age.

Experimental design

Throughout the experiments, Day 0 means the day of egg inoculation and all the litters in each separate strains of mice were divided at random into two groups. In Experiment 1, there were Cortisone group and Non-treated group. Mice of Cortisone group were treated with cortisone acetate (2.5 or 5.0 mg/day; Cortone, 25 mg/ml, Nippon Merk-Banyu Co. Ltd., Japan) twice on Day −1 and Day 0 (2 hr prior to egg inoculation). All the mice were given an identical inoculation with the same batches of shell-free eggs (5,000 or 10,000) on Day 0, and killed 4 days after egg inoculation. In Experiment 2, there were Day 12 group and Day 15 group. All the mice were given an identical inoculation with 100 shell-free eggs on Day 0. Mice of Day 12 and Day 15 groups were killed on 12 days and 15 days after egg inoculation, respectively. In Experiment 3, there were Egg group which was given 100 shell-free eggs on Day 0, and Cysticercoid group which was given 100 cysticercoids on Day 5, and a number of mice in both groups were killed simultaneously on Days 12, 15, 20 or 30.

Assay of H. nana *infection*

In Experiment 1, cysticercoid recovery in the intestinal wall was examined by Hunninen's (1935) method, and cysticercoids were searched also at the liver surface with the naked eye and under a microscope.

In Experiments 2 and 3, in order to recover worms the intestine was cut open lengthwise carefully not to damage the worms and placed in a 15 cm diameter Petri dish containing 0.85% NaCl solution. Worms were removed after an examination with the naked eye, and soon afterward stored in a 3 cm diameter Petri dish containing 0.85% NaCl solution. Within 1 hr they were weighed after removing surface liquid on filter paper (= fresh biomass). After weighing of fresh biomass, these worms were completely teased apart and washed through a stainless sieve upon a 10 cm diameter round bottomed glass dish. All eggs released through this procedure including eggs released in the Petri dish were carefully collected into a 30 ml test tube. The egg suspension of 30 ml was kept undisturbed at room temperature for 1 hr, then 20 ml of the supernatant was decanted off. Counting of the eggs was done soon thereafter or after keeping them at 4 C overnight. Immediately after the egg suspension of 10 ml had been stirred completely on a thermomixer, the number of mature eggs described by Moriyama (1961) was counted in three samples of 0.05 ml, each of which was dropped on a slide glass and covered with a cover glass of 24×50 mm. When the eggs counted in each sample were more than 500, a ten-fold dilution was performed. Counting was carried out under ×100 magnification.

Results

Effect of cortisone acetate on cysticercoid recovery

Experiment 1. This experiment was designed to investigate whether cortisone affects the host's susceptibility to *Hymenolepis nana* infection. Both dd and BALB/c mice were used. The details of the experimental design and the results are summarized in Table 1: Although the cortisone doses used were sufficient to make the hosts immunosuppressed (Okamoto, 1969; Ito and Yamamoto, 1977), there was no significant difference in the number of cysticercoids recovered. In both BALB/c and dd mice either treated with cortisone or not, about 20% of shell-free eggs administered developed into cysticercoids in the intestinal tissue, and a half of animals

Table 1 Comparison of the host susceptibility to a primary infection with *Hymenolepis nana* eggs between dd and BALB/c strains of mice

Mouse strain	Cortisone treatment (Day-1 and Day 0)	Egg dose (Day 0)	No. of mice infected	No. of cysticercoids in the villi Mean±S.D. (Range)
dd	2.5 mg×2	5,000	5/5	1,072±191.5 (842–1269)
dd	NT	5,000	5/5	999±163.8 (757–1161)
BALB/c	2.5 mg×2	5,000	4/4	1,006± 79.5 (947–1116)
BALB/c	NT	5,000	5/5	1,065±162.8 (787–1189)
dd	5.0 mg×2	10,000	5/5	1,989±265.5 (1,600–2214)
dd	NT	10,000	5/5	2,034±236.0 (1,775–2312)
BALB/c	5.0 mg×2	10,000	5/5	2,054±134.0 (1,894–2198)
BALB/c	NT	10,000	5/5	2,104±286.9 (1,725–2374)

The cortisone doses used have been proven to be sufficient to suppress the host immune responses to reinfection (Okamoto, 1969; Ito and Yamamoto, 1977).

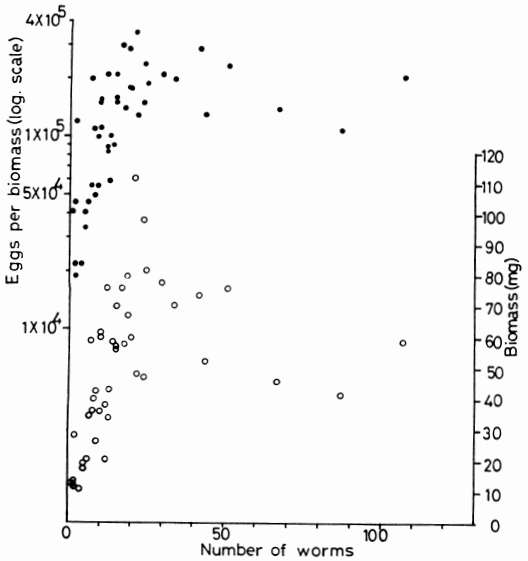

Fig. 1 Correlation between egg output (●) or biomass (○) and worm numbers in cysticercoid-derived, 15-day-old *Hymenolepis nana* recovered from dd strain of mice.

given 1×10^4 shell-free eggs harbored a few numbers of cysticercoids in ectopic liver tissue.

Different figures of fecundity of H. nana

In a preliminary work by the use of dd mice which had been given cysticercoids, it was found that establishment of 20 worms/mouse showed the highest fecundity as illustrated in Fig. 1.

Experiment 2. Whether dd and BALB/c mice given an identical infection with 100 shell-free eggs of the same batch become to harbor similar numbers of adult worms or not was examined by measuring: 1) the numbers of adult worms established, 2) the fresh biomass, and 3) the output of mature eggs, during the earlier days of the patency (Days 12 and 15) (Table 2).

Both strains of mice harbored similar numbers of worms within Day 15, but the output of mature eggs and the fresh biomass

Table 2 Comparison of the growth of *Hymenolepis nana* of a primary infection between dd and BALB/c strains of mice given 100 shall-free eggs of the same batches on Day 0

Day of observation	Mouse strain	No. of mice infected	No. of worms recovered Mean±S.D. (range)	Mean No. of eggs produced	Mean biomass
Day 12	dd	10/10	23.1±7.71 (12–38)	5.6×10^4 eggs	46.0 mg
Day 12	BALB/c	10/10	22.0±7.01 (11–32)	1.1×10^4	17.5
Day 15	dd	10/10	24.3±9.90 (13–45)	2.4×10^5	85.8
Day 15	BALB/c	10/10	22.9±8.99 (14–44)	2.8×10^4	24.4

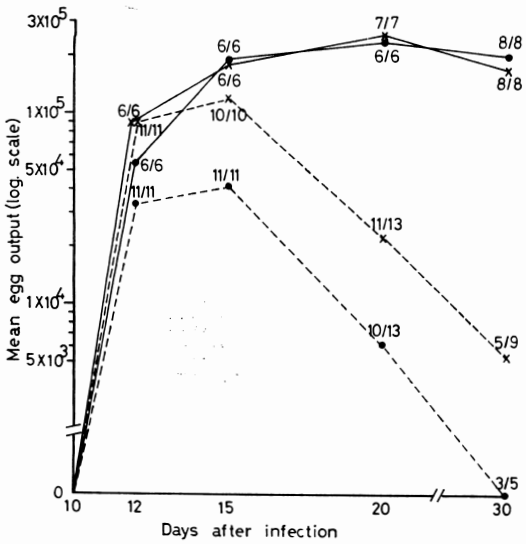

Fig. 2 Mean egg output during infection with eggs (●) or cysticercoids (×) of *Hymenolepis nana* in two different strains of mice (——, dd mice; - - -, BALB/c mice). The number of mice found infected on each day is indicated.

of each worm population clearly differed between these two strains of mice throughout the experiment. Worms recovered from dd mice produced much more mature eggs and biomass than those from BALB/c mice at Day 12 (5.6×10^4 eggs vs. 1.1×10^4 eggs; 46.0 mg vs. 17.5 mg) and greatly increased their fecundity by Day 15 (from 5.6×10^4 to 2.4×10^5 eggs; from 46.0 to 85.8 mg), whereas those recovered from BALB/c mice increased a little in egg output and their biomass (from 1.1×10^4 to 2.8×10^4 eggs; from 17.5 to 24.4 mg). Clearly from the start of the patency, worms recovered from dd mice were much more fecund than those recovered from BALB/c mice which were given eggs, although no egg output started within Day 10 in either strain mice.

Experiment 3. This experiment was designed to determine whether or not cysticercoid-derived worms (CdW) become more fecund than egg-derived worms (EdW). The results are illustrated in Fig. 2. As a quantitative index of the fecundity, the egg output was compared as shown in Fig. 2. Both EdW and CdW recovered from dd mice showed a similar pattern in their fecundity. A plateau phase in the egg output was reached within Day 15 and continued till Day 30, the last day of the experiment. In contrast, EdW and CdW recovered from BALB/c mice showed completely different figures, as clearly shown in Fig. 2, and the fecundity was much less than that which were recovered from dd mice: In BALB/c mice the egg output from CdW was greater than that from EdW, but no plateau phase was reached. CdW of Day 12 only showed the same fecundity as CdW recovered from dd mice. Both EdW and CdW established in dd mice survived till Day 30, whereas those in BALB/c mice began to decline rapidly in worm load between Days 15 and 20. In addition, seven of the eight dd mice of Day 30, which had been given cysticercoids, were found autoinfected, whereas none of BALB/c were reinfected.

Discussion

The results described above present an evidence supporting the previous observation that the longevity and fecundity of *Hymenolepis nana* grown in BALB/c mice was much shorter and lower than that in dd mice (Ito, 1980). The obvious difference in the mode of the primary infection of *H. nana* found at least between these two strains of mice is primarily attributed to the difference in the rapidity of onsets of the acquired immune responses directed against the lumen phase of *H. nana*, which has been found previously (Ito, 1980, 1981), but not to the difference in the host's susceptibility, or innate immunity to this parasite infection.

To some other metacestode infections cortisone increases the host's susceptibility not only in resistant strains but also in the so-called susceptible strains of mice (re-

viewed by Mitchell, 1979; Wakelin, 1978) and results in an obvious increase in cysticercus load. However, in the present results, cortisone doses used, which has been found to be sufficient to suppress the early response, or the well-known rapid resistance to egg challenges of *H. nana* (Okamoto, 1969; Ito and Yamamoto, 1977), did not affect cysticercoid load in the intestinal wall nor in the ectopic liver tissue at all. The obvious difference in influence of cortisone, assessed by the number of bladder worms, upon the host's susceptibility to *H. nana* and to other metacestodes might be primarily due to the difference in the length of time required to larval maturation, as previously speculated by Brown (1976) or due to the difference in the life cycle *per su*. Immunosuppression depresses both innate and acquired immunity. This affects establishing metacestodes of Taeniidae and results in obvious increase in cysticercus load (Kamiya *et al.*, 1980; Mitchell *et al.*, 1977; Olivier, 1962). In contrast, it can't affect cysticercoids of *H. nana* (Isaak *et al.*, 1977; Reed *et al.*, 1977), since the late response directed against cysticercoid challenge is only acquired after the target, cysticercoids have successively grown up adult worms (Ito, 1980). Therefore, it seems probable that in *H. nana* infection in mice, innate immunity, or susceptibility is distinguishable from acquired immunity.

When a great number of shell-free eggs (such as 1×10^4 or more) were administered, only a small number of the cysticercoids (less than 1% of cysticercoids recovered) were found at the liver surface, but almost all cysticercoids were formed in the intestinal wall, as previously reported (Astafiev, 1966; Inoue *et al.*, 1979). At this point, most recent work by Lucas *et al.* (1980) seems very interesting, since CBA mice treated with cortisone harbored a number of cysticercoids, most of which enlarged and showed abnormality, in the liver. However, the present results showed no obvious difference in the frequency of the cysticercoid emergence at the liver surface between mice treated with cortisone and those nontreated, within 4 days of egg inoculation, at least by the use of dd and BALB/c mice. Further, it has been found that almost all established cysticercoids of a primary infection develop into adult worms in dd mice (Ito and Yamamoto, 1976), in BALB/c mice (Isaak *et al.*, 1977) and in other strains (Hearin, 1941). Isaak *et al.* (1977) clearly demonstrated that there is no difference in the number of cysticercoids or adults of a primary infection recovered from immunologically normal mice and congenitally athymic immunodeficient mice. Therefore, it seems probable that both dd and BALB/c mice, at least, are similarly susceptible to *H. nana* infection.

Previous works (Isaak *et al.*, 1977; Ito, 1980) have demonstrated that *H. nana* changes its immunogenicity during the course of development and stimulates stage specific responses; *i.e.* the early response, directed against egg challenges, the late response against cysticercoid challenges, and worm expulsion response against the initially established adult worms themselves. In dd mice both EdW and CdW similarly matured and no difference in fecundity was observed throughout as previously suggested (Ito and Yamamoto, 1977), whereas both EdW and CdW recovered from BALB/c mice showed obviously different figures (Fig. 2). The different figures between these two strains of mice seem to be well agreed with the different figures of the rapidity of onsets of the late response and the worm expulsion response (Isaak *et al.*, 1977; Ito, 1980).

On the other hand, CdW were always slightly more fecund than EdW only when recovered from BALB/c mice. Similar results by the use of CD1 mice given beetle-derived cysticercoids and eggs have been reported previously by Ghazal and Avery (1974). They explained the difference due

to the onset of the early response. However, more recently it has been found, as mentioned above, that the early response is directed exclusively against the oncosphere of egg challenge, but never against cysticercoid challenges (Ito, 1980). Further, Ito (1981) has demonstrated that at least in BALB/c mice, cysticercoid inoculation may induce the late response exclusively without stimulating the early response and result in the failure of autoinfection, and a tissue phase of egg inoculation is not always necessary for initiation of the late response but is for that of the early response.

It may be, therefore, probable to conceive that the somewhat different (but basically similar) figures between CdW and EdW recovered from BALB/c mice may be explained as follows: Either 1) the early response somewhat enhances or accelerates the onset of the late response, as previously speculated (Ito, 1980) or 2) not only the cysticercoids in the lumen but also those formed in the intestinal tissue may serve to stimulate the late response. The latter appears more likely, although highly speculative.

Summary

Cortisone acetate (5.0 mg or 10 mg/mouse) did not affect cysticercoid recovery in a primary infection with *Hymenolepis nana* eggs in both BALB/c and dd strains of mice, even when egg doses were increased up to 10,000 shell-free eggs. No difference in the host's susceptibility to the tissue phase of *H. nana* infection existed at least between these two strains of mice. Nevertheless the fecundity of the established *H. nana* became obviously different throughout: In dd mice, a plateau phase in the egg output was reached within 15 days of egg inoculation, whereas in BALB/c mice no plateau phase was reached. The different figures of *H. nana* infection between these two strains of mice are discussed in terms of the difference in onset of the immune responses directed against the lumen phase of *H. nana, i.e.* the late response against cysticercoid challenges and the worm expulsion response against the established worms themselves.

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BALB/c, dd 2系統マウス群間での小形条虫感染像の比較

伊藤　亮
(岐阜大学医学部寄生虫学教室)

BALB/c, dd 2系統マウスで観察される小形条虫初感染虫体の成熟度に著しい差異が認められるため，本虫感染に対する感受性（先天免疫）および（再）感染防御獲得免疫の発現時期の2点を検討すべく実験を行なった．その結果，両系統マウス群間で擬嚢尾虫寄生数とその多寡におよぼすコーチゾンの影響は認め得なかった．その反面，成熟期初期虫体の湿重量，子宮内感染型虫卵数には著しい差異が観察され，dd マウス寄生虫体の方が BALB/c マウス寄生虫体より明らかに成熟度が高かった。次に虫卵投与群と擬嚢尾虫投与群とにおけるこれらの動態を30日目まで追跡比較した結果，BALB/c マウスでのみ両群間に若干の差が認められた．BALB/c マウスの擬嚢尾虫投与群から得られた虫体は上述の虫卵投与群から得られた虫体より虫体成熟度が明らかに高く，12日令虫体は dd マウス寄生の同日令虫体と差がなかった．しかし15日令虫体で若干の虫卵数増多が認められた後急激な虫卵数減少と虫体消失が観察された．対照的に dd マウスでは両群間で虫卵数に全く差がなく，15日令虫体の虫卵数は12日令虫体の2倍に増え，その後30日令まで殆ど変化なく，虫卵数減少，虫体消失は全く観察されなかった．以上の成績は BALB/c, dd 2系統マウス群間で観察される本虫初感染像の著しい差異が両系統間の本虫に対する感受性あるいは先天免疫の差異によるのではなく，既報のごとく本虫感染後獲得される（再）感染防御免疫の発現時期の差異によることを示唆する．