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Immunization of Rats Against *Mesocestoides corti* (Cestoda) by Subcutaneous Vaccination of Living Tetrathyridia and by Passive Transfer With Serum

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ABSTRACT

Laboratory rats were subcutaneously vaccinated with 100 live tetrathyridia of *Mesocestoides corti* (Cestoda) and subsequently intraperitoneally challenged with 50 tetrathyridia. Necropsy 30 days postinfection revealed that vaccinated rats harbored 97.4% fewer worms compared to control rats. In a second experiment, passive transfer of immunity was accomplished by immune serum from subcutaneously vaccinated rats. Rats receiving immune serum harbored 33.4% lighter worm burdens compared to normal serum recipients.

INTRODUCTION

Research on cestode immunology has focused on the relatively few tapeworms with mammalian tissue-invading phases in their larval development. The great majority of research has centered on *Taenia* Linnaeus and *Hymenolepis* Weinland, possibly due to their economic importance and the fact that both genera are relatively easy to maintain in the laboratory. Moreover, some species belonging to these genera are pathogenic parasites of humans and domestic animals. Therefore, a certain amount of economic impetus has accounted for much of the immunological research regarding these tapeworms.

Protection against *Mesocestoides corti* infections has been accomplished by vaccination of mice with excretory-secretory (ES) products collected from tetrathyridia incubated in vitro and by passive transfer of serum from chronically infected mice (Kowalski and Thorson 1972a, 1972b). Kazacos (1976) successfully immunized mice against *M. corti* by subcutaneous inoculation of live tetrathyridia 21 days prior to intraperitoneal challenge infections.

As the relative rates of *M. corti* tetrathyridial proliferation are considerably lower in rats than mice (Specht and Voge 1965) it was of interest to investigate the responses of rats to immunization procedures and to compare those results with the previously mentioned studies performed with mice.

MATERIALS AND METHODS

Stock infections

Rats (Wooster-Ohio strain) were obtained from the Department of Animal Science, University of Arkansas. Stock infections were maintained in young rats (6-8 weeks old). Tetrathyridia were washed in three rinses of Kreb's ringer solution (pH 7.2) prior to intraperitoneal (IP) injection with a sterile syringe fitted with a 21-gauge needle. Worms were harvested from 30-day infections and used in subcutaneous (SC) vaccination procedures and in intraperitoneal (IP) challenge infections.

Preparation of immune serum

Thirty rats (6-8 weeks old) were SC vaccinated with 100 tetrathyridia according to the procedures of Kazacos (1976). Serum was collected and pooled 21 days subsequent to SC vaccination.

Collection of serum

Blood was collected from control and SC vaccinated rats by cardiac puncture using a sterile syringe fitted with a 21-gauge needle. Blood was allowed to clot in sterile glass test tubes overnight at 2°C. Sera from each treatment group were pooled, centrifuged (2200 rpm/10 min), and frozen until used. No preservatives were added to any serum samples.

Subcutaneous vaccination and challenge infections

Fifteen rats (6-8 weeks old) were SC vaccinated with 100 tetrathyridia as previously described. Twenty-one days later 15 SC vaccinated rats and 15 control rats were IP infected with 50 tetrathyridia using a sterile syringe fitted with a 21-gauge needle. Rats were necropsied 30 days post-infection following the procedures of Kowalski and Thorson (1972a). Worms were fixed in 10% formalin prior to making direct worm counts of each rat's worm burden.

Passive transfer of serum and challenge infections

Thirty rats (6-8 weeks old) were IP infected with 50 tetrathyridia as previously described. Within 2 hours each rat received an IP injection of either 2.0 ml of pooled immune serum or pooled normal serum. Thirty days postinfection rats were necropsied and direct worm counts were made as before.

Statistical analysis

Student's t-test was employed to determine the statistical significance between the worm burdens from treatment and control groups. A probability value of 0.05 or less was considered significantly different.

RESULTS

Subcutaneously vaccinated rats were highly resistant to IP challenge infections, demonstrating a 97.4% average reduction in worm burdens compared to control rats (Table 1). Moreover, 46.7% of the SC vaccinated rats demonstrated a "sterile immunity" (i.e., no worms were recovered). Livers were infected in only 26.7% of the SC vaccinated rats.

Passive transfer of immunity using pooled serum from SC vaccinated rats resulted in a 33.4% average reduction in worm burdens compared to rats receiving normal serum (Table 1). Liver necropsies revealed exceptionally light worm burdens in all 15 rats receiving immune serum.

DISCUSSION

Subcutaneous vaccination of rats with live tetrathyridia produced a high degree of protection to IP challenge infections. Kazacos (1976) observed a 43.9% average reduction in worm burdens in mice SC vaccinated and IP challenged with tetrathyridia of *Mesocestoides corti*. As the rate of tetrathyridial proliferation is considerably slower in rats than mice, it might be assumed that the "immuno-physiological" mechanisms of rats have a greater retarding effect on tetrathyridial proliferation than those mechanisms in mice. The results of the present study suggest that prior SC vaccination augmented this process which resulted in significantly lower worm burdens and in some cases, a "sterile immunity". It should be noted that in SC vaccinated rats tetrathyridia were actually eradicated rather than just suppressed from asexually reproducing. In contrast, Kazacos' (1976) study of SC vaccinated mice revealed that tetrathyridia continued to asexually proliferate, although at decreased rates.

As would be expected, passive transfer of pooled immune serum was less effective in protection than SC vaccination. Pooled immune serum recipients demonstrated a 33.4% average decrease in worm

burdens compared to rats receiving normal serum. In contract, Kowalski and Thorson (1972a) demonstrated a 78% average reduction in *M. corti* worm burdens following passive transfer of immune serum. It should be noted that they utilized serum from chronically infected mice and the challenge infection period was 10 days while a 30-day infection period was used in the present study.

The extremely low rate of tetrathyridial proliferation in rats necessitated a 30-day challenge infection period in the present study. A greater reduction in worm burdens following serum treatment might have been observed if rats were necropsied 10 days postinfection. However, preliminary studies of rats harboring 10-day infections indicated great disparities of worm burdens within treatment groups.

The fact that SC vaccination elicited a greater degree of protection than immune serum suggests that other immunological factors might augment humoral immune mechanisms against *M. corti* infections in rats.

The complexities of cestode immunology remain to be completely elucidated. The unique biological characteristics of the *Mesocestoides corti* tetrathyridium make it an ideal model for such investigations.

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Table 1. Effects of subcutaneous vaccination and passive transfer of serum on intraperitoneal challenge infections with tetrathyridia of *Mesocestoides corti* in rats.

No. of rats	Treatment	No. of tetrathyridia in IP challenge	No. of tetrathyridia recovered 30 days postinfection: Mean (standard error)	No. of tetrathyridia recovered 30 days postinfection: Total (range)	Percent reduction compared to controls
15	subcutaneous vaccination	50	2.8* (±0.88)	42 (0-20)	97.4
15	untreated controls	50	115.7 (±2.47)	1,646 (97-128)	_____
15	2.0 ml immune serum	50	74.7* (±3.08)	1,120 (49-90)	33.4
15	2.0 ml normal serum	50	112.2 (±3.51)	1,683 (91-131)	_____

* Significantly different from controls, $P<0.05$