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Veterinary Parasitology 74 (1998) 179–189

veterinary parasitology

Depressed immunity to a *Salmonella typhimurium* vaccine in mice experimentally parasitized by *Taenia crassiceps*

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Received 8 May 1997; accepted 8 September 1997

Abstract

To assess the immunological status of mice parasitized with *Taenia crassiceps* metacestodes, 6-month old female BALB/c mice experimentally parasitized with *T. crassiceps* and immunized with *Salmonella typhimurium* antigens were infected with *S. typhimurium* virulent bacilli ($1.6 \times LD_{50}$). Both *T. crassiceps*-parasitized and immunized and parasitized mice showed a very high susceptibility to infection ($^{**}P < 0.01$) with higher bacteremia than control and immunized-control animals and produced a reduced IgG response to *S. typhimurium* antigens ($^{*}P < 0.05$). This indicates that *T. crassiceps* is able to preclude development of immunity to *S. typhimurium*, because appropriate antibody production to a heterologous antigenic stimulus did not take place, and the bacteremia results suggest the parasitosis altered the mononuclear phagocyte system. It has been demonstrated that *Taenia solium* metacestodes produce a small RNA molecule in culture which suppresses humoral and cellular responses against homologous antigens in mice. We propose that *T. crassiceps* may be actively synthesizing such a factor, apart from other simultaneously acting immunomodulatory mechanisms, to induce an immunosuppressed state favorable to its development in the host. © 1998 Elsevier Science B.V.

Keywords: *Taenia crassiceps*; Cestoda; Cysticercosis; *Salmonella typhimurium*; Infection; Immunomodulation

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PII S0304-4017(97)00173-8

1. Introduction

Experimental murine infection by *Taenia crassiceps* (Freeman, 1962) generates a chronic and massive cysticercosis where parasite burden sometimes equals the host's mass. Clinically, the parasitized mice seem quite healthy in laboratory conditions and necropsy does not show major pathology other than localized inflammation in the peritoneal cavity. Good and Miller (1976) reported depression of primary and secondary humoral responses to sheep erythrocytes in mice implanted with metacestodes of *T. crassiceps*. Other studies have shown depression of (^{3}H) thymidine incorporation in lymphocytes from mice infected with *Taenia taeniaeformis*, *T. crassiceps* or implanted with *Taenia solium* metacestodes (Hammerberg and Williams, 1978; Leid et al., 1984, 1986; Letonja et al., 1987; Willms et al., 1980; Sciutto et al., 1995) and in lymphocytes from naturally cysticercotic pigs stimulated with Concanavalin A (ConA) (Molinari et al., 1993). Moreover, T cell proliferation and IL-2 production in response to ConA or anti-CD3 stimulation were significantly depressed in mice infected with *T. crassiceps* (Sciutto et al., 1995). As has been shown in other experimental models of parasitism, *T. crassiceps* infection results in a Th1–Th2 imbalance favoring Th2 (Terrazas et al., 1994; Villa and Kuhn, 1996), a situation which in some instances has been correlated with the host's susceptibility to parasitic infection (Finkelman et al., 1991; Cox and Liew, 1992).

Inhibition of lymphocyte proliferation by low molecular weight factors produced by *Schistosoma mansoni* schistosomula, *Onchocerca gibsoni* microfilariae, *T. taeniaeformis* larvae, and *T. solium* metacestodes in vitro has been reported (Dessaint et al., 1977; Yin Foo et al., 1983; Leid et al., 1984, 1986; Burger et al., 1986; Molinari et al., 1990; Tato et al., 1995). A low molecular weight metacestode factor (MF) obtained from *T. solium* has been shown to suppress immunity to *Salmonella typhimurium* antigens in immunized mice (Molinari et al., 1989). This substance has been characterized as a small RNA-peptide (1450 Da) which has been shown to depress lymphocyte proliferation (Molinari et al., 1990; Tato et al., 1995) and to exert an inhibitory effect either on inflammation around implanted *T. solium* metacestodes in mice or on humoral and cellular immunity (Tato et al., 1996). We speculated *T. crassiceps* infected mice might likewise show a depressed immunity to a *Salmonella* infection, since this parasite might also be actively synthesizing immunodepressing factors such as MF.

The present study was conducted to determine how mice parasitized with *T. crassiceps* and immunized with *S. typhimurium* antigens respond to an infectious challenge with virulent *S. typhimurium* bacilli.

2. Materials and methods

The fast growing *T. crassiceps* strain ORF was kindly supplied by Dr. C. Larralde (Instituto de Investigaciones Biomédicas, UNAM). Since 1986 parasites have been maintained by sequential intraperitoneal (i.p.) inoculation of metacestodes in female BALB/c mice as reported by Freeman (1962). Metacestodes for experimental infection were obtained from female donor mice infected 3–6 months previously. Groups of six 4–6 week-old female BALB/c inbred mice maintained at our animal facilities, were

infected using a 25-gauge needle with 10 non-budding, approx. 2 mm in diameter *T. crassiceps* metacestodes. The parasitized mice in the following experiments had been infected 12 weeks with *T. crassiceps* and then challenged with virulent *S. typhimurium* bacilli. Controls were always litter or age mates. The average parasitic load of a parallel control group of female BALB/c mice at 12 weeks of *T. crassiceps* infection was determined by opening the peritoneal cavity and counting the number of cysts found inside.

A strain of *S. typhimurium* kindly given by Dr. L. Filloy (Pediatric Hospital of Mexico) was cultured in CASOY broth and identified by agglutination with *Salmonella* O antigen B group antiserum (Difco). *S. typhimurium* antigens were prepared by the bacterial acetone powder method as described by Tato et al. (1979). Protein concentration of the antigens was determined by the method of Lowry et al. (1951). Each mouse was inoculated subcutaneously (s.c.) with 50 μg of protein (*Salmonella* antigens) resuspended in 100 μl sterile 0.9% saline solution, 20 and 10 days prior to the challenge with *S. typhimurium* bacilli. This corresponds approximately to 9 and 10.5 weeks of *T. crassiceps* infection. Median lethal doses (LD_{50}) were estimated according to Reed and Muench (1938).

Lightly anesthetized female mice were infected intragastrically with 1×10^6 *S. typhimurium* bacilli which were obtained during their midlog growth phase, as described by Tato et al. (1979). Bacteremia were determined as described by Molinari et al. (1989). Briefly, 10 μl of blood were obtained from the tail vein of each animal at intervals of 72 h, starting 1 day after challenging and lasting through the 21st day. Blood was obtained under sterile conditions with graduated micropipettes, and was immediately mixed with 4 ml 1% soft brilliant green agar (Merck) maintained at 40°C, stirred vigorously for 5 s and plaqued on brilliant green agar in duplicate petri dishes. The cultures were incubated at 37°C for 24 h. The colonies were counted, averaged, and expressed as colony-forming units (CFU). Mortality was registered daily during 21 days of observation and results were plotted as percent survival.

ELISA serum antibody determinations were performed as described by Coligan et al. (1991), using *Salmonella* antigens as substrate. Western blots (WB) were performed with 8% SDS–PAGE using a *S. typhimurium* antigen concentration of 90 mg/cm^2, and transferred to pure nitrocellulose membranes (0.2 μm) in a semi-dry transfer cell (Bio-Rad), following the method of Towbin et al. (1979), using pre-stained molecular weight standards (Bio-Rad).

S. typhimurium infection, mortality and CFU determinations were performed at the same time every morning; animals that died shortly after the CFU determination were considered to have survived up to that day. Mortality rates were analyzed by Fisher's exact test and ELISA results by Student's *t*-test.

3. Results

T. crassiceps-infected female BALB/c mice presented a mean ($\pm$SEM) parasitic load of 1061 ($\pm$457) metacestodes at 12 weeks of infection.

A dose response curve of inoculum concentrations showed that 1×10^6 *S. ty-*

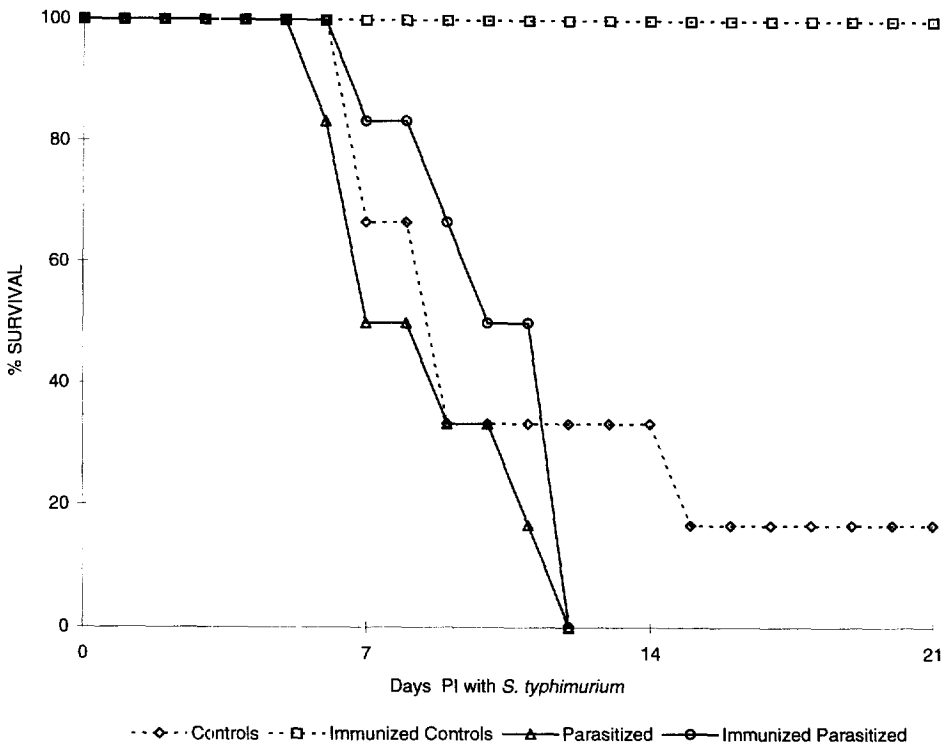

Fig. 1. Mortality curves of control and *T. crassiceps* parasitized female mice after *S. typhimurium* intragastrical infection ($^{**}P < 0.01$). Both Immunized Control and Immunized Parasitized mice were vaccinated twice with *S. typhimurium* antigens, 20 and 10 days prior to *S. typhimurium* challenge.

phimurium bacilli ($1.6 \times LD_{50}$) was the optimal dose for our purposes (data not shown). The intragastrical infection with virulent bacilli ($1.6 \times LD_{50}$) of parasitized mice previously immunized against *S. typhimurium* clearly showed the inability of these animals to respond with an effective protective immunity against this challenge. None of the immunized-control mice died post-infection (PI) with 1×10^6 *S. typhimurium* bacilli at 3 weeks, whereas 16.6% of controls survived this period (Fig. 1, $n = 6$, $^{**}P < 0.01$). In contrast, mortality in both *T. crassiceps*-parasitized and immunized and parasitized mice reached 100% within less than 2 weeks.

Bacteremia determinations (CFU) showed higher mean values in parasitized than in control mice (Table 1, $n = 6$). Table 1 shows both the individual and the surviving animals' average CFU values obtained throughout the experiment, in order to illustrate the temporal development of *S. typhimurium* infection in a comparative manner. Except for the case of one immunized control, once bacteremia results were positive, the animals died within a few days (range 1–10, mean 3.11). CFU values did not always represent the actual bacteremia at the time of death, but some data were obtained just prior to the animal's death (as indicated by an asterisk in Table 1). Interestingly,

Table 1
Bacteremia after *S. typhimurium* infection

Days of survival PI	Controls						Average	Immunized controls						Average
	1	2	3	4	5	6		1	2	3	4	5	6	
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	1	1	0	0	1	0	0.50	0	0	0	0	0	3	0.50
6	92	50	13	0	0	0	25.83	0	0	0	0	0	600	100.00
8			2128[a]	1	0	0	532.25	0	0	0	0	0	100	16.67
10					2	0	1.00	0	0	0	0	0	0	0
12					1	0	0.50	0	0	0	0	0	0	0
14					7	0	3.50	0	0	0	0	0	0	0
16						0	0	0	0	0	0	0	0	0
19						0	0	0	0	0	0	0	0	0
21						0	0	0	0	0	0	0	0	0

Days of survival PI	Parasitized						Average	Immunized parasitized						Average
	1	2	3	4	5	6		1	2	3	4	5	6	
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	6	20	0	0	0	0	4.33	9	1	1	0	0	0	1.83
6	ND	2000[a]	6	9	0	1	403.20	12000[a]	1	0	3	0	0	2000.67
8				28000[a]	20	2	9340.67		412	0	34	0	0	89.20
10					274	76	175.00				27	81	0	36.00
12						ND							1[a]	1
14														
16														
19														
21														

PI = Post-infection.
CFU = Colony-forming units.
ND = Not determined since the animal died the same day prior to blood extraction.
Blank spaces indicate the animals had already died.
[a]Indicates these animals were bled and died shortly later the same day.

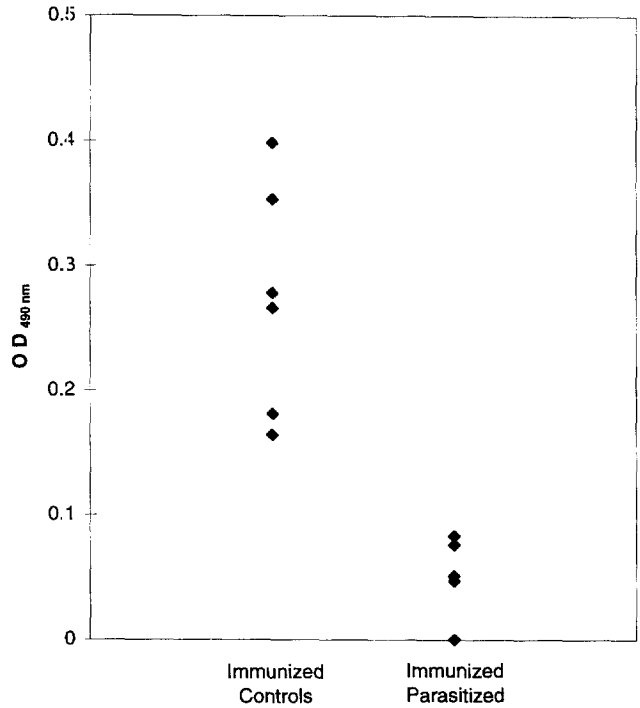

Fig. 2. Total IgG antibodies against *S. typhimurium* antigens produced by Immunized Control and Immunized Parasitized mice, as determined by ELISA ($^{*}P < 0.05$).

parasitized mice had higher values than immunized and parasitized ones, suggesting the vaccine did offer some protection, albeit not enough to prevent death.

When measured by ELISA, IgG antibodies to *S. typhimurium* antigens in immunized and parasitized mice were inhibited significantly when compared to immunized control mice (Fig. 2, $n = 5$, $^{*}P < 0.05$). Preliminary Western Blot data indicated that immunized-control mice consistently recognized 4 *S. typhimurium* antigens (3 above 200 kDa, and 1 between 36 and 27 kDa), whereas immunized and parasitized animals did not (data not shown).

4. Discussion

Female BALB/c mice parasitized by *T. crassiceps* and immunized with *S. typhimurium* antigens exhibited an immunosuppressed state when challenged with virulent *S. typhimurium* bacilli. Three parameters support this assertion.

(a) Both *T. crassiceps*-parasitized, and immunized and parasitized mice died soon after the infectious challenge ($1.6 \times LD_{50}$) and mortality rose to 100% at day 12 in both groups. In contrast, 100% of the immunized-control mice survived 21 days of observation ($^{**}P < 0.01$).

(b) Bacteremia rates were highest in parasitized animals, followed by the immunized and parasitized group (Table 1). The control group showed moderate CFU and only one immunized-control mouse exhibited a level of bacteremia that was lethal for mice in the other groups, which was nevertheless completely controlled by this mouse by the 10 day PI.

(c) Parasitized mice did not respond to the antigenic stimulus (*Salmonella* antigens) as did the immunized-control mice ($^{*}P < 0.05$).

Immunity against *S. typhimurium* is controlled by an early nonspecific immunity involving macrophages (Edwards et al., 1991), while late immunity is considered to be serotype-specific and T cell mediated (Tite et al., 1991). The switch from early to late immunity against this bacteria is mediated by Th1 cytokines, such as tumor necrosis factor (TNF) (Tite et al., 1991) and interferon-gamma (IFN-γ) (Nauciel and Espinasse-Maes, 1992). Experiments with innately susceptible BALB/c mice involving oral challenge with salmonellae have shown that both immune serum and T cells are required for protective immunity and it is suggested that antibodies by themselves can increase the level of nonspecific resistance, although they are not capable of restraining nor aborting the intracellular growth of virulent bacteria (Mastroeni et al., 1993). This study demonstrates that mice parasitized by *T. crassiceps* and immunized with *S. typhimurium* antigens are not capable of producing an effective immune response against a *S. typhimurium* infection.

The immune response of a given host can become 'locked' in either the Th1 or Th2 direction (Williams et al., 1992). Chronic infection resulting in long term exposure to the parasite's antigens in high concentration can be regarded as a kind of hyperimmunization, and could lead to a 'locked' Th2 immunity (Caulada-Benedetti et al., 1991; Finkelman et al., 1991). Indeed, *T. crassiceps* infection has been shown to induce a Th1–Th2 imbalance favoring Th2 (Terrazas et al., 1994; Villa and Kuhn, 1996). This immunity, apparently 'locked' in anti *T. crassiceps* humoral immunity, may not be able to effectively produce antibodies to other concomitant stimuli. A similar phenomenon has been reported in murine *Mesocestoides corti* infection (Abraham and Teale, 1987), where the parasite somehow restricts the isotypes being produced.

A Th2 polarized response leads to a preferential production of the IgG1 over the IgG2a isotype. *T. crassiceps* infection results in Th2 immunity, and can be considered to present an {IgG1/IgG2a} ratio that reflects the relative dominance of Th2 over Th1, although this has not yet been demonstrated. *S. typhimurium* killed vaccines have been shown to induce a Th2 response in BALB/c mice (Harrison et al., 1997), and this effect is not qualitatively dependent on the route of immunization (Thatte et al., 1995). We therefore assume that immunization with *S. typhimurium* antigens does not revert, nor substantially modify, the Th2 response previously elicited by *T. crassiceps* infection. The observed changes in anti-*S. typhimurium* IgG responses between the *T. crassiceps*-parasitized and control groups is thus considered not to be a reflection of differences in IgG subclasses.

The fact that parasites can alter a host's capacity to develop protective immunity has been described in children vaccinated against *S. typhi* after acute *Plasmodium falciparum* malaria who showed depressed immunity against the bacterial antigens, as determined by antibody titre (Williamson and Greenwood, 1978). This was one of the

first results pointing to this protozoan causing an immunosuppressive state that has since been extensively documented (Sztein and Kierszenbaum, 1993). An helminth, *S. mansoni*, has been found to alter the host's capacity to develop effective immunity after vaccination: *S. mansoni*-infected patients, particularly those with hepatointestinal disease, showed a diminished ability to mount an immune response to *S. typhi* after the immunization with a typhoid vaccine (Muniz-Junqueira et al., 1996).

A metacestode factor (MF) obtained from *T. solium* induced suppressed immunity in mice immunized with *Salmonella* antigens and challenged with virulent *S. typhimurium* bacilli (Molinari et al., 1989) and had a dose-dependent depressive effect on (^{3}H) thymidine uptake by phytohaemagglutinin-stimulated cultured human lymphocytes (Molinari et al., 1990). This substance has been characterized as a small RNA molecule with a molecular weight slightly above 1450 Da and the capacity to inhibit (^{3}H) thymidine uptake by murine lymphocytes in vitro (Tato et al., 1995). Taeniids have already been reported to produce immunomodulatory molecules (Hammerberg and Williams, 1978; Leid et al., 1986; Laclette et al., 1992; White et al., 1992a), and the suggestion of such a moiety produced by *T. crassiceps* is reasonable. Immunosuppression induced by *T. solium* is an active process, since dead metacestodes are known to elicit the host's inflammatory response, while this does not happen as long as the cysticerci are intact (White et al., 1992b). In our laboratory, we have observed inhibition of humoral and cellular responses to *T. solium* metacestode antigens in mice treated with a RNA-peptide MF, and also inhibition of inflammation around *T. solium* metacestodes implanted in these mice (Tato et al., 1996). Recently, we have observed that *T. solium* MF impairs the production of IL-2, IL-4, IFN-γ and TNF-α (Arechavaleta et al., 1997). *T. crassiceps* might similarly be actively producing such an immunodepressive factor, as occurs in other parasites (Dessaint et al., 1977; Yin Foo et al., 1983; Burger et al., 1986; Tato et al., 1995, 1996). This factor could act simultaneously with other proposed mechanisms, such as host hormonal feminization and Th1–Th2 imbalance induction (Terrazas et al., 1994; Larralde et al., 1995; Villa and Kuhn, 1996), to achieve an immunosuppressed state favorable to its development in the host.

The addition of recombinant IL-2 to cell cultures partially restored responsiveness (Sciutto et al., 1995), which seems to indicate immunosuppression is relevant in generating a host environment favorable to the parasite. Our results show that experimental *T. crassiceps* cysticercosis causes systemic immunomodulation affecting the host's IgG humoral and possibly cellular immunity, and thus increases susceptibility to infection, as against *S. typhimurium* infection. However, under laboratory conditions, parasitized animals do not show other syndromes associated with a generalized immunosuppression, such as opportunistic infections or neoplasias, a fact that indicates the parasite-induced modifications to the host's immune system do not exceed a critical threshold that would be detrimental for its own permanence in the host. This, of course, does not necessarily apply to natural conditions, which may be far more diverse and challenging than the experimental laboratory conditions.

T. crassiceps induces immunosuppressive mechanisms that affect fundamentally the host's effective immunity against the parasite itself and *S. typhimurium*, showing that an immune response to an infectious agent can predispose the host to increased susceptibility to a second one (Wassom, 1993). We propose that *T. crassiceps* might be actively

producing an immunosuppressive factor which, in concert with other proposed modulatory mechanisms, accounts for the depressed immunity against *S. typhimurium* infection. To our knowledge, this is the first report of *T. crassiceps* infection resulting in an alteration to the host's ability to produce heterologous antibodies, which indirectly points to an alteration of the Th2, as well as the Th1 T cell subsets.

Acknowledgements

We thank Dr. Carlos Larralde for his support and ideas for the manuscript, and Yazmín de la Garza for technical assistance. A different report of these experiments was submitted by M.R. as a BSc thesis (Universidad Nacional Autónoma de México, 1994) and supported by a scholarship from DGAPA/UNAM.

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