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Studies of macrophage function during *Trichinella spiralis* infection in mice

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Summary. Studies were made to investigate the quantitative and functional changes which occur in peritoneal macrophage populations obtained from mice infected orally with *Trichinella spiralis* larvae. C57BL/6 mice infected with *T. spiralis* larvae became parasitized with adult worms which were rejected from the intestine from 14 to 20 days after infection. Infected mice developed a striking increase in peritoneal exudate cells, composed largely of macrophages, which was maximal at from 16 to 18 days after infection. *T. spiralis* larvae and eosinophils were not seen in the peritoneal exudates. Macrophages from mice infected more than 11 days earlier inhibited DNA synthesis of syngeneic and allogeneic tumour cells, a property attributed to activated macrophages. In addition, macrophages from *T. spiralis*-infected mice had the functional ability to kill EL-4 tumour cells as measured by ^{51}Cr release. Unlike activated macrophages, however, macrophages from infected mice did not develop the ability to inhibit multiplication of the intracellular pathogen *Toxoplasma gondii*. These studies demonstrate that *T. spiralis* infection in mice induces changes in macrophage function that differ from changes associated with infections by intracellular pathogens.

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INTRODUCTION

Whereas macrophages have been shown to play a central role in host resistance to intracellular pathogens such as *Listeria monocytogenes* (Mackaness, 1962) and *Toxoplasma gondii* (Ruskin, McIntosh & Remington, 1969), their role in infections caused by other types of pathogens has been less well defined. Resistance to certain helminthic infections including the intestinal stage of *Trichinella spiralis* (Larsh, Race, Goulson & Weatherly, 1966; Love, Ogilvie & McLaren, 1976) has been shown to be mediated by cellular immunity, but the role of macrophages and alterations in macrophage functions during these helminthic infections have not been systematically studied. As an initial step in defining the role of macrophages in risistance to *T. spiralis* infection in mice, we have investigated change in effector functions of macrophages during this infection.

MATERIALS AND METHODS

Mice

C57BL/6 female mice weighing 16–18 g were obtained

from Jackson Laboratory, Bar Harbor, Maine. In each experiment, mice were matched for age.

Infection with T. spiralis and T. gondii

The strain of *T. spiralis* was kindly provided by Dr John Larsh, University of North Carolina, Chapel Hill, North Carolina. Mice were infected with *T. spiralis* as previously described (Larsh & Kent, 1949; Weatherly, 1970). The number of adult worms in the gut of infected mice was determined by the method of Larsh & Kent (1949). Chronic *T. gondii* infection in mice was established by intraperitoneal injection of 1×10^5 C56 strain *T. gondii* and subsequent treatment with sulfadiazine (Ruskin *et al.*, 1969).

Preparation of macrophages

Peritoneal exudate cells (PEC), used as a source of macrophages, were harvested and processed as previously described (Wing & Remington, 1977). PEC from immunized animals were from mice infected with either *T. spiralis* 3 to 37 days earlier, *T. gondii* 1·5 to 6 months earlier, or injected intraperitoneally with 1400 μg of killed *Corynebacterium parvum* (supplied by Wellcome Research Laboratories, Beckenham, Kent, United Kingdom) 7 days previously. For each experiment, *T. spiralis* macrophages were compared with normal macrophages and macrophages activated by *T. gondii* infection or *C. parvum* injection (which inhibit and kill both tumour cells and *T. gondii*) (Hibbs, Lambert & Remington, 1972; Remington, Krahenbuhl & Mendenhall, 1972; Olivotto & Bomford, 1974; Ghaffar, Cullen, Dunbar & Woodruff, 1974; Swartzberg, Krahenbuhl & Remington, 1975). After the cells were harvested, they were brought to an appropriate concentration in medium 199 (Grand Island Biological Co., Grand Island, New York) containing 10% heat-inactivated foetal calf serum (Grand Island Biological Co., Grand Island, New York), penicillin 100 u/ml and streptomycin 100 μg/ml (M199-FCS).

PEC and macrophages from normal, *T. spiralis*-infected and *T. gondii*-infected mice were counted and examined morphologically using Wright's stain and Giemsa stain; the percentage of phagocytic cells was determined using heat-killed *Candida albicans* or iron filings. The percentage of PEC that were adherent to plastic was determined by counting the number of cells plated.

Tumour inhibition assay

Macrophages obtained by plating 9×10^5 PEC per well in 6 mm Linbro tissue culture plates were challenged with 2×10^4 tumour cells (either syngeneic EL-4 cells or allogeneic P815 cells) in 0·2 ml of M199-FCS as previously described (Gardner & Remington, 1977; Wing, Gardner, Ryning & Remington, 1977). A cytostatic index (CI) was defined as $(N-A)/N \times 100$ where N = counts per minute (c.p.m.) of tumour cells plus normal macrophages and A = c.p.m. of tumour cells plus macrophages from immunized mice. Statistical evaluation was by the Student's *t* test.

Tumour cytotoxicity assay

The assay for *in vitro* release of ^{51}Cr from target cells was adapted from the methods used by Cerottini & Brunner (1971). Briefly EL-4 cells were labelled with [^{51}Cr]-sodium chromate (specific activity 100–300 Ci/mM, New England Nuclear, Gardena, California). The cells were suspended (10^5 cells/ml) in Dulbecco's medium (Grand Island Biological Company, Grand Island, New York) plus penicillin 100 u/ml, streptomycin 100 μg/ml, and 10% heat-inactivated foetal calf serum and plated on macrophage monolayers (2×10^6 PEC/well). Total amount of cell- associated ^{51}Cr was measured, and the amount of ^{51}Cr which was releasable (75%) was determined by lysing labelled EL-4 with distilled water. Twenty-four hours after challenge, the amount of ^{51}Cr released into the supernatant in the presence of macrophages was assayed. Percentage of ^{51}Cr released was determined by the formula:

$$\frac{\text{Experimental release} - \text{Spontaneous release}}{\text{Total releasable} - \text{Spontaneous release}} \times 100$$

where experimental release is the amount of ^{51}Cr released from cultures of target cells cultured in the presence of normal or activated macrophages, spontaneous release is the amount released from target cells alone, and total releasable is the amount of ^{51}Cr released by distilled water.

Assay of intracellular multiplication by T. gondii

Macrophages (from 1×10^6 PEC) were prepared in 4-chambered Lab Tek slides and then challenged with 2×10^6 RH strain *T. gondii* trophozoites in M199-FCS for 1 h as previously described (Anderson & Remington, 1974). It was previously shown in our laboratory that macrophages from normal mice support intracellular multiplication by *T. gondii* trophozoites, and macrophages from *T. gondii*-infected mice do not (Remington *et al.*, 1972). The percentage of infected

cells with multiplying *T. gondii* and the number of *T. gondii* per infected vacuole were determined for 100 infected cells in each chamber.

RESULTS

Enumeration of adult *T. spiralis* in the intestine

To determine when adult *T. spiralis* are rejected from the intestine, mice were infected orally with *T. spiralis* larvae. At various times after infection, groups of four mice were killed, and the number of adult worms in the gut counted. A representative experiment in which mice were infected with 288 larvae is shown in Table 1

Table 1. The number of adult *T. spiralis* in the gut of mice infected with 288 *T. spiralis* larvae

Days after infection with *T. spiralis* larvae	Mean number of adult *T. spiralis* per mouse (± SEM)*
8	77 (±9·5)
12	69 (±13)
14	65 (±18)
20	5 (±2·3)
22	0·3 (±0·3)
27	0

* Mean for four mice for each time period.

(similar results were obtained in eight separate experiments). The maximum number of adult worms in the gut was 28% of the number of larvae used for infection. This percentage varied with each experiment (range 20–39%). The number of adult worms remained constant until day 14, after which there was a great decrease in the worm load. All worms were eliminated by 27 days after infection.

Quantitative and qualitative changes in peritoneal exudate cells following infection with *T. spiralis*

Mice infected with 530 *T. spiralis* larvae had increased numbers of cells in their peritoneal cavities (Fig. 1). The maximum number of cells (32×10^6/mouse) was reached from 16 to 18 days after infection which temporally coincided with a decrease in the number of adult worms in the intestine (Table 1). These results were reproducible in eight experiments with infections of 200 to 530 larvae.

In separate experiments, PEC from mice at 7 and 37 days after parasite infection contained more than 99% mononuclear cells, rare basophils and neutrophils; no

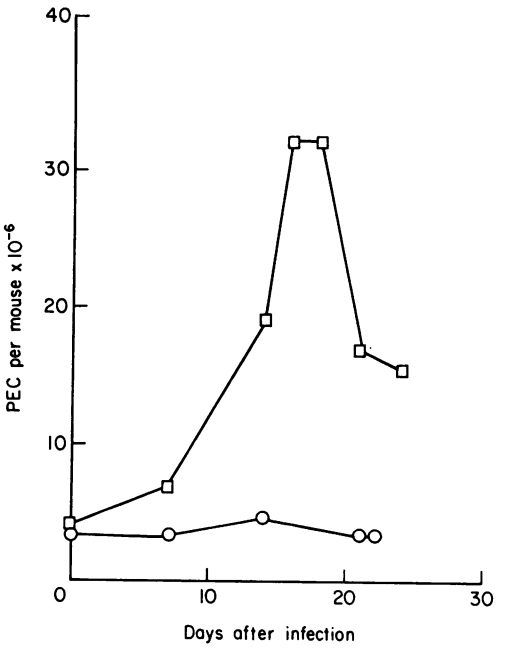

Figure 1. Change in the number of PEC after oral infection with 530 *T. spiralis* larvae. (o) Normal; (□) *T. spiralis*.

eosinophils, and no *T. spiralis* larvae were seen. Between 50 and 60% of PEC from normal mice and from mice infected at various times after *T. spiralis* or *T. gondii* infection phagocytosed iron filings or heat-killed *Candida albicans*. The mean percentage of adherent cells in populations of PEC obtained from normal mice and *T. spiralis*-infected mice were found to be 49% (range 45–52%) and 45% (range 35–54%) respectively. These percentages were not significantly different ($P > 0·2$) and were similar to those found with *T. gondii*-infected mice (45–50%). More than 99% of the adherent PEC from normal, *T. spiralis*-infected, and *T. gondii*-infected mice were mononuclear and more than 90% were phagocytic.

Effect of macrophages from *T. spiralis*-infected mice on DNA synthesis of EL-4 tumour cells

Results of a representative experiment designed to determine whether macrophages from *T. spiralis*-infected mice have the capacity to inhibit incorporation of [^{3}H]-TdR into DNA of EL-4 tumour cells are shown in Fig. 2. No significant difference was noted between the effect of macrophages from normal and *T. spiralis*-infected mice until 11 days after infection at which time macrophages from *T. spiralis*-infected

mice inhibited incorporation of radiolabel into tumour cells by more than 90% ($P < 0.001$ for each comparison more than 11 days after infection) as shown by the CI. This capacity to inhibit tumour cells persisted for 29 days after infection in the experiment shown in Fig. 2; in other experiments (Table 2) the capacity to inhibit tumour cells decreased after 2 weeks. This capacity of macrophages from *T. spiralis*-infected mice to inhibit tumour cells was noted in more than twenty experiments utilizing five separately infected groups of mice, each of which had received from 200 to 530 lavae. Similar results were noted when P815 tumour cells were used as target cells.

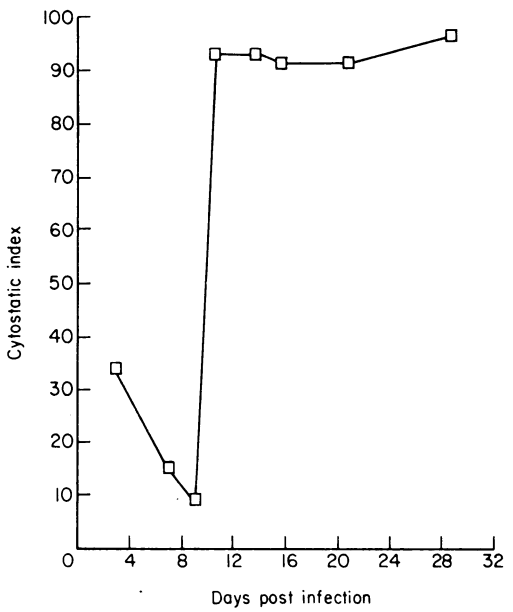

Figure 2. Inhibition of EL-4 tumor cell [^{3}H]-TdR uptake by macrophages from *T. spiralis*-infected mice.

Effect of macrophages from *T. spiralis*-infected mice on intracelluar multiplication of *T. gondii*

To determine the effect of macrophages from *T. spiralis*-infected mice on multiplication of an intracellular pathogen, macrophage monolayers from these mice

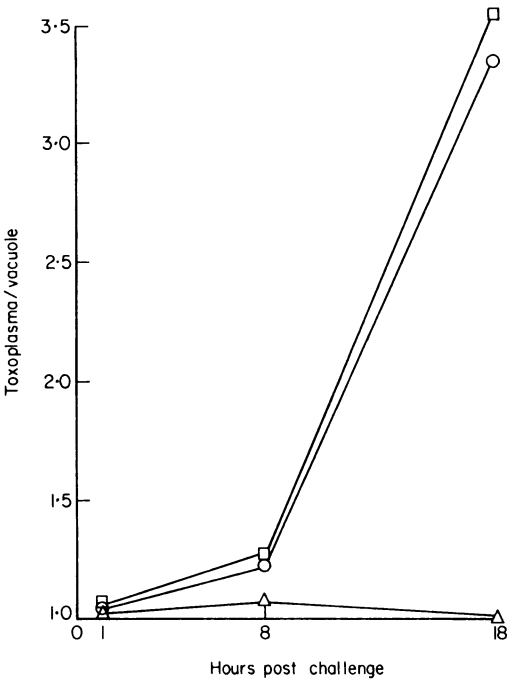

Figure 3. Effect of macrophages from *T. spiralis*- and *T. gondii*-infected mice and normal mice on intracellular multiplication of *T. gondii*. Macrophage monolayers from *T. spiralis*- and *T. gondii*-infected mice and normal mice were challenged with *T. gondii* and intracellular multiplication was measured by methods previously described (Remington *et al.*, 1972; Anderson & Remington, 1974). ○, Normal; □, *T. spiralis*; △, *T. gondii*.

Table 2. Effect of macrophages from normal, *T. spiralis*-infected, and *T. gondii*-infected mice on multiplication of *T. gondii* and tumour cell DNA synthesis

Days post-infection with *T. spiralis*	Macrophage effect on: *T. gondii* Source of macrophage			Tumour cell (CI) Source of macrophage	
	Normal	*T. spiralis*	*T. gondii*	*T. spiralis*	*T. gondii*
7	3·1 (98)*	2·8 (96)	1·0 (2)	15	≥98
14	2·7 (96)	2·6 (98)	1·0 (0)	98	≥98
21	3·2 (99)	2·9 (97)	1·0 (1)	83	≥98
37	3·3 (98)	3·1 (98)	1·0 (0)	59	≥98

* Figure outside parenthesis = No. toxoplasma/vacuole. Figure inside parenthesis equals percentage infected macrophages with toxoplasma which had multiplied.

Table 3. Cytotoxic capacity of macrophages from *T. spiralis*-infected mice*

Source of macrophages	^{51}Cr† Release	[^{3}H]-TdR uptake Inhibition (CI)	*T. gondii*
Normal mice	−7·4	—	6·03 (99%)‡
C. parvum-injected mice	+51·1	80·1	1·33 (33%)
T. spiralis-infected mice	+50·1	98·8	5·71 (100%)

* All determinations made at 24 h.

† ^{51}Cr release = $\frac{\text{Experimental release} - \text{Spontaneous release}}{\text{Total releasable} - \text{Spontaneous release}}$

‡ Figure outside parenthesis = No. toxoplasma/vacuole. Figure inside parenthesis equals percentage infected macrophages with toxoplasma which had multiplied.

were challenged with RH strain *T. gondii* and examined for intracellular multiplication after 1, 8, 18 h. Results of a representative experiment shown in Fig. 3 reveal that macrophages from both uninfected mice and *T. spiralis*-infected mice supported intracellular multiplication of *T. gondii*, whereas macrophages from mice chronically infected with *T. gondii* did not. Macrophages of mice infected with challenges of from 200 to 530 larvae and obtained from 7 to 37 days after infection allowed multiplication of *T. gondii* (Table 2). Macrophages from mice chronically infected with *T. gondii*, however, inhibited both tumour cells and multiplication of *T. gondii*.

Effect of macrophages from *T. spiralis*-infected mice on the viability of EL-4 tumour cells as measured by ^{51}Cr release

Experiments were carried out to determine whether macrophages from *T. spiralis*-infected mice had the functional capacity to kill EL-4 tumour cells. Macrophages were harvested from either normal mice, *T. spiralis*-infected mice which were infected with 271 larvae 14 days earlier, or *C. parvum*-injected mice. ^{51}Cr release, inhibition of [^{3}H]-TdR uptake, and inhibition of *T. gondii* multiplication were determined. Results are shown in Table 3. Macrophages from *T. spiralis*-infected mice and *C. parvum*-injected mice inhibited [^{3}H]-TdR uptake by EL-4 tumour cells and also had a cytocidal effect as determined by ^{51}Cr release; normal macrophages did not.

DISCUSSION

Our results demonstrate several features of peritoneal macrophage kinetics and function during *T. spiralis* infection in mice. We consistently observed a considerable increase in the number of peritoneal exudate cells in mice after oral infection with *T. spiralis*. This increase in cells, as high as eight times the number of cells found in normal mice, coincided with elimination of adult worms from the gut. Previous workers have shown that macrophages and lymphocytes infiltrate the intestinal wall at the time of immune rejection of adult worms from the gut (beginning approximately 11 days after *T. spiralis* infection in mice) (Larsh & Race, 1975). Whether changes in PEC numbers which we observed reflect inflammatory changes in the intestinal wall remains to be investigated.

Our results differ from those of Pelley, Karp, Mahmoud & Warren (1976), who found that PEC from Swiss mice infected 4 weeks previously with 270 *T. spiralis* larvae contained 40–70% eosinophils. These investigators, however, injected proteose peptone solution into the peritoneal cavities of mice 48 h prior to harvesting peritoneal cells, a procedure which may have drawn circulating eosinophils into the peritoneal cavity. Our studies differ from this in that no foreign material was injected into the peritoneal cavities of our animals.

In the studies on the functional properties of macrophages, we noted that those obtained from animals infected with *T. spiralis* significantly inhibited incorporation of [^{3}H]-TdR into syngeneic and allogeneic tumour cells. One mechanism to explain the inhibitory effect of macrophages on EL-4 tumour cells was elucidated by Stadecker, Calderon, Karnovsky & Unanue (1977), while our experiments were in progress. Stadecker and his colleagues demonstrated that DNA synthesis and cell proliferation by EL-4 tumour cells

may be inhibited by thymidine which is sythesized and subsequently released into the medium by macrophages. Our results show that macrophages from *T. spiralis*-infected mice have the functional capacity to kill EL-4 tumour cells as measured by the ^{51}Cr release assay. This cytocidal effect was confirmed in parallel experiments using [^{3}H]-TdR release as a measure of cell killing (unpublished results). Because of the capacity of *T. spiralis* macrophages both to kill EL-4 cells and to inhibit P815 tumour cells (a tumour cell line less sensitive to thymidine [Stadecker *et al.*, 1977]), release of thymidine by *T. spiralis* macrophages and its subsequent inhibitory effect on tumour target cells is probably not responsible for the effects we observed.

The nonspecific capacity of macrophages to inhibit tumour cells has been demonstrated for macrophages obtained from animals infected with intracellular pathogens (Hibbs *et al.*, 1972) and injected with a variety of substances such as killed *C. parvum* (Olivotto & Bomford, 1974; Ghaffar *et al.*, 1974), Pyran copolymer (Kaplan, Morahan & Regelson, 1974), and proteose peptone (Keller, 1973). Functions of macrophages from animals with helminthic infections, however, have been incompletely characterized. An *in vitro* study by Keller & Jones (1971) suggested that macrophages from rats infected with *Nippostrongylus brasiliensis* ingested and decreased the viability of Walker carcinosarcoma cells *in vitro*. A more recent study by Meerovitch & Bomford (1977) documented the capacity of macrophages from *T. spiralis*-infected CD1 mice to inhibit mouse R1 leukemia cells *in vitro*. Macrophages from *T. spiralis*-infected mice inhibited leukemia cells as early as 6 days after infection but lost this capacity by 36 days.

When we studied a second function of macrophages from *T. spiralis*-infected mice, a remarkable dichotomy was noted (Wing *et al.*, 1977). It is known that activated macrophages, e.g. those obtained from mice infected with *L. monocytogenes* (Mackaness, 1962) or *T. gondii* (Remington *et al.*, 1972) inhibit *in vitro* multiplication of intracellular pathogens including *L. monocytogenes* (Mackaness, 1962), *T. gondii* (Remington *et al.*, 1972), and *Salmonella typhimurium* (Blanden, 1968). 'Activated' macrophages from *T. spiralis*-infected mice, however, were unable to prevent intracellular multiplication of *T. gondii* and did not differ in this respect from macrophages of normal mice.

This dissociation between inhibition and killing of tumour cells and inhibition of an intracellular pathogen is unique. One possible explanation is that a subpopulation of macrophages which has the potential to inhibit and kill tumour cells is activated by *T. spiralis* infection but that a second separate subpopulation which has the potential to inhibit intracellular pathogens is not activated by *T. spiralis* infection. Neither subpopulation would be activated in normal mice and both would be activated by chronic *T. gondii* infection. Evidence to support the existence of separate functional subpopulations of macrophages has been reported by several investigators. Walker (1976) was able to separate rabbit peritoneal exudate cells on discontinuous ficoll gradients and noted that cytotoxicity of the macrophages for chemically induced adenocarcinoma cells varied within the macrophage subclasses. Rice & Fishman (1974) separated peritoneal exudate cells from rabbits on continuous or discontinuous albumin gradients and found differences in macrophage production of immunogenic RNA, in endocytic capacity and in morphology. Lee & Berry (1977) separated mouse macrophages by velocity sedimentation and observed that immunostimulatory cells could be separated from tumour-suppressive cells. Similarly, results from our laboratory revealed that varying immunization schedules with certain pathogens allowed dissociation of effector functions of macrophages (Wing *et al.*, 1977). A second explanation for the observed dissociation of functions is that the two functions of activated macrophages we studied require different degrees of activation. This hypothesis predicts that *T. spiralis* infection is a less potent activator of macrophages than chronic *T. gondii* infection. Whether either of these hypotheses is valid remains to be clarified.

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