EFFECTS OF TOXOPLASMA GONDII (GLEADLE STRAIN) ON THE HOST-PARASITE RELATIONSHIP IN TRICHINOSIS

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Abstract—COPELAND D. and GROVE D.I. 1979. Effects of Toxoplasma gondii (Gleadle strain) on the host-parasite relationship in trichinosis. International Journal for Parasitology 9: 205–211. The effects of concurrent infection with Toxoplasma gondii on the host-parasite relationship in trichinosis were studied. Infected mice showed a delay in expulsion of Trichinella spiralis adults from the gut. Persisting adult female worms were fecund but the numbers of larvae recovered from the muscles were not increased. Increased resistance to the systemic phase of trichinosis was shown by reduced numbers of muscle larvae after intravenous injection of newborn larvae in animals with toxoplasmosis as compared with control mice. There were no differences in small bowel pathology of trichinous mice with and without toxoplasmosis but inflammation around muscle cysts of T. spiralis was reduced in mice with toxoplasmosis. The eosinophilia which normally develops in mice with trichinosis was suppressed by concurrent toxoplasmosis. Trichinella infection did not alter the numbers of T. gondii cysts recovered from the brain 4 weeks after infection. It is suggested that the delay in expulsion of adult worms, decrease in muscle inflammation around T. spiralis cysts, and inhibition of eosinophilia result from immune suppression, while the reduction in numbers of muscle larvae after intravenous injection of newborn larvae reflects enhanced nonspecific resistance to infection in toxoplasmosis.

INDEX KEY WORDS: Toxoplasma gondii; Trichinella spiralis; mice; intestinal expulsion; fecundity; muscle larvae; resistance; partial villous atrophy; muscle inflammation; eosinophilia.

INTRODUCTION

Infection with the intracellular protozoon, Toxoplasma gondii, has diverse effects on the host's immune system. Infected mice become immunosuppressed with impairment of antibody formation (Strickland, Voller, Pettit & Fleck, 1972; Strickland, Pettit & Voller, 1973), inhibition of splenic plaque-forming cells (Strickland & Sayles, 1977), suppression of lymphocyte stimulation by T and B cell mitogens (Strickland, Ahmed & Sell, 1975) and prolongation of allograft rejection (Hibbs, Lambert & Remington, 1973). Furthermore, toxoplasmosis in experimental animals is associated with lymphocytopenia (Huldt, 1966), reduction in thymus weight and loss of cortical thymocytes (Huldt, Gard & Olovson, 1973).

On the other hand, infection with T. gondii confers a remarkable nonspecific resistance to infection with phylogenetically unrelated organisms such as viruses (Remington & Merigan, 1969), bacteria (Ruskin & Remington, 1968), fungi (Gentry & Remington, 1971) and helminths (Mahmoud, Warren & Strickland, 1976). It is thought that this heightened resistance is due to activation of macrophages by persistent, intracellular Toxoplasma organisms (Ruskin, McIntosh & Remington, 1969; Remington, Krahenbuhl & Mendenhall, 1972).

There is, therefore, a dichotomy between resistance to infection and immunological competence in animals with toxoplasmosis (Remington & Krahenbuhl, 1976). We have investigated the effects of T. gondii on the host-parasite relationship in trichinosis, an infection which is itself associated with immunosuppression (Svet-Moldavsky, Shaghjian, Chernyakhovskaya, Mkheidze, Litovchenko, Ozeretskova & Kadaghidze, 1970; Faubert & Tanner, 1971; Cypess, Lubiniecki & Hammon, 1973; Faubert & Tanner, 1974; Ljungstrom & Huldt, 1977). Finally, the effect of Trichinella spiralis on toxoplasmosis has been also briefly studied.

MATERIALS AND METHODS

Animals. Outbred female Swiss albino mice (CF1), 18–20 g in weight, were obtained from Carworth Farms, Inc. (New City, NY, U.S.A.).
Parasitological techniques. T. gondii cysts were obtained from animals infected more than 6 weeks previously with the Gleadle strain. This strain was originally isolated from human cervical lymph node tissue and causes an avirulent infection in mice (Strickland et al., 1972). The brains were removed and macerated in phosphate buffered saline (PBS). Experimental animals were infected intraperitoneally with 10 cysts suspended in 0·2 ml brain emulsion to which penicillin (100 units/ml) and streptomycin (10 μg/ml) were added. Similar volumes of brain emulsion from uninfected mice containing antibiotics were injected intraperitoneally into control animals. T. gondii infection was quantitated in experimental animals by removal of brains 4 weeks after infection, maceration in PBS, followed by counting the number of cysts in aliquot samples and calculation of the number of cysts per brain. A colony of infected mice was established from a strain of T. spiralis originally supplied by Dr. W. C. Campbell (Merck Institute for Medical Research, NJ, U.S.A.). Larvae were isolated by maceration of carcasses in a Waring Blender, incubation in 1% acid–pepsin for 2–3 h at 37°C, sedimentation in saline and filtration through gauze (Gould, 1970). Mice were infected with 300 larvae in 0·2 ml of saline by oesophageal intubation with a 16 gauge needle. Adult worms were recovered from the small intestines by a modification of the method of Campbell (1967). The small bowel was removed, slit longitudinally, cut into 2–3 cm pieces and digested in 100 ml of 0·01 M-NaOH and 0·15 M-saline overnight at 4°C. The intestinal fragments were shaken vigorously and poured into a Petri dish, the pieces removed and the fluid transferred to 50 ml centrifuge tubes. The worms were spun down at 400 g for 5 min and the total number in the sediment counted. The number of larvae in the muscle of each carcass was counted. Each animal was decapitated, skinned, eviscerated, macerated and digested for 4 h at 37°C in 1% acid–pepsin in a total volume of 100 ml. Each digestate was thoroughly shaken, 0·2 ml samples were taken, and the number of larvae counted in duplicate. Newborn larvae of T. spiralis were obtained using a modification of the method of Dennis, Despommier & Davis (1970). Thirty mice were each infected with 800 larvae by oesophageal intubation with a 16 gauge needle. Adult worms were recovered from the small intestines by a modification of the method of Dennis, Despommier & Davis (1970). The right hamstring muscles were removed, fixed in 10% formalin, sectioned and stained with haematoxylin and eosin. The villus : crypt ratio of jejunal mucosa was determined by measurement of villi and crypt heights in 10 representative, well-orientated villi in at least two separate portions of each biopsy (Roberts-Thomson, Grove, Stevens & Warren, 1976). The right hamstring muscles were removed, fixed in 10% formalin, sectioned and stained with haematoxylin and eosin. The areas of inflammation around 10 isolated larvae cut, longitudinally, but not including the larvae themselves, were measured with a πMC particle measurement computer system (Millipore Corp., Bedford, MA, U.S.A.) (Grove & Warren, 1976). Absolute counts of eosinophils were made from blood samples obtained in the morning from the retro-orbital plexus with a microhaematocrit tube and diluted in Discomb's fluid (one part acetone, one part 2% aqueous eosin and eight parts distilled water).

RESULTS

Intestinal worm burdens

Thirty-two mice were infected with T. gondii. Ten days later, these animals together with the same number of control mice were infected with 300 larvae of T. spiralis. Intestinal worm burdens were determined 1, 2, 3 and 5 weeks later (Fig. 1). There were no significant differences between the two groups at one week. By two weeks after infection, much worm expulsion has taken place in the control mice as is shown by the worm burden falling to 47% of the value on day 7. In mice infected with T. gondii, however, no significant change had occurred; the difference between the two groups was statistically significant (P < 0·001, 't' test). Three weeks after infection, worm expulsion had commenced in mice infected with T. gondii, the worm burden being 70% of the value on day 7, but recovery of worms from the control group had fallen further to 11% of the initial level (P < 0·001, 't' test). Five weeks after infection, very few worms were present in either group of mice.

In a second experiment, 10 mice were infected with T. gondii. Four months later, these animals together with the same number of control mice were infected with 300 larvae of T. spiralis. There were no significant differences between the two groups on assay 16 days later, the worm counts being 5·8 ± 1·8 S.E.M. and 9·4 ± 1·7 S.E.M. respectively.
Concurrent toxoplasmosis and trichinosis

Fig. 1. The numbers of adult worms recovered from the gut at various intervals after infection of mice with 300 larvae of *T. spiralis*. ○—○, control mice; ●—●, mice injected intraperitoneally with 10 cysts of *T. gondii* 10 days prior to infection. Eight mice were in each group.

**Muscle worm burdens**

Sixteen mice were infected with *T. gondii*. Ten days later, these animals together with the same number of control mice were infected with 300 larvae of *T. spiralis*. The numbers of muscle larvae were counted 3 and 5 weeks after infection. There were no significant differences between the two groups. Three weeks after infection, 35,900 ± 4700 S.E.M. larvae were recovered from mice infected with *T. gondii* and 35,800 ± 5250 S.E.M. larvae from control animals. The corresponding values five weeks after infection were 86,500 ± 14,500 S.E.M. and 69,000 ± 8500 S.E.M. larvae.

**Fecundity**

Mice were infected with *T. gondii* then 10 days later, these animals and control mice were infected with *T. spiralis*. One week after this infection, adult worms were recovered and their fecundity was measured. There were no significant differences between the two groups: worms recovered from 8 control mice produced 54 ± 6 S.E.M. newborn larvae/female worm/24 h, while those obtained from 8 mice infected with *T. gondii* produced 56 ± 5 newborn larvae/female worm/24 h. When worms were recovered from mice infected with *T. gondii* 16 days after infection with *T. spiralis*, they were still fecund, producing 57 ± 7 S.E.M. newborn larvae/female worm/24 h. Only a small number of worms could be recovered from control animals at this time; they averaged 7 newborn larvae/female worm/24 h.

**Resistance to newborn larvae**

Ten mice were infected with *T. gondii*. Ten days later, these animals, together with the same number of control mice were injected with 650 newborn larvae of *T. spiralis* intravenously. Four weeks later, muscle larval counts were performed. There was a significant 48% reduction in the number of muscle larvae in mice infected with *T. gondii* compared with control mice (Table 1).

**Small bowel pathology**

When compared with normal uninfected mice, there was a moderate reduction in the villus : crypt ratio of intestinal biopsies from both mice infected with *T. gondii* and from control mice when they were examined 1, 2 and 3 weeks after infection with 300 larvae of *T. spiralis* (Table 2). By 5 weeks after infection, the villus : crypt ratio had almost returned to normal. There were no significant differences between the two groups of mice at any time.

**TABLE 1—RECOVERY OF *T. spiralis* MUSCLE LARVAE FROM NORMAL MICE AND FROM MICE INFECTED WITH *T. gondii* 4 WEEKS AFTER INTRAVENOUS INJECTION OF 650 NEWBORN LARVAE**

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>Mean ± S.E.M.</th>
<th>Probability*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control mice</td>
<td>10</td>
<td>380 ± 25</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><em>T. gondii</em>-infected mice</td>
<td>10</td>
<td>200 ± 29</td>
<td></td>
</tr>
</tbody>
</table>

*'t' test.

**TABLE 2—THE VILLUS : CRYPT RATIO [MEAN ± S.E.M.] OF THE SMALL BOWEL AT VARIOUS INTERVALS AFTER INFECTION OF MICE WITH 300 LARVAE OF *T. spiralis***

<table>
<thead>
<tr>
<th>Weeks after infection</th>
<th><em>Trichinella</em> only</th>
<th><em>Toxoplasma</em> plus <em>Trichinella</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.37 ± 0.18</td>
<td>2.42 ± 0.10</td>
</tr>
<tr>
<td>2</td>
<td>2.17 ± 0.12</td>
<td>2.20 ± 0.45</td>
</tr>
<tr>
<td>3</td>
<td>2.34 ± 0.08</td>
<td>Not done</td>
</tr>
<tr>
<td>5</td>
<td>3.00 ± 0.11</td>
<td>2.77 ± 0.11</td>
</tr>
</tbody>
</table>

One group of mice was infected with *T. gondii* 10 days before exposure to *T. spiralis*. Eight mice were in each group. The mean value for normal, uninfected mice is 3.1 ± 0.1 S.E.M.
Muscle inflammation

Mice were infected with *T. gondii* then, 10 days later, these animals together with the same number of control animals were infected with 300 larvae of *T. spiralis*. There was a significant suppression of inflammation 3 and 5 weeks after infection with *T. spiralis* in those animals which had also been infected with *T. gondii* (Table 3).

### Table 3—The area of inflammation around muscle larvae (Mean ± S.E.M.) in *μ*l after 3 and 5 weeks after infection with 300 larvae of *T. spiralis*.

<table>
<thead>
<tr>
<th>Weeks after infection</th>
<th>Trichinella only</th>
<th>Toxoplasma plus Trichinella</th>
<th>Probability*</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>7700 ± 460</td>
<td>3700 ± 360</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>5</td>
<td>10,700 ± 830</td>
<td>6000 ± 820</td>
<td>&lt; 0.005</td>
</tr>
</tbody>
</table>

*’t’ test.

One group of mice was infected with *T. gondii* 10 days prior to exposure to *T. spiralis*. Seven or eight mice were in each group.

Eosinophil levels

Mice were infected with *T. gondii* then, 10 days later, these animals together with the same number of control mice were infected with 300 larvae of *T. spiralis*. Absolute eosinophil levels were determined for the next 5 weeks (Fig. 2). Control mice developed the normal eosinophilia (Grove, Mahmoud & Warren, 1977) between 2 and 4 weeks after infection with *T. spiralis*. This eosinophilia was suppressed, however, in animals which were also infected with *T. gondii* (*P* < 0.005, < 0.02 and < 0.025 at 2, 2.5 and 3 weeks respectively, ‘*t’* test).

**Effect of *T. spiralis* infection on Toxoplasma numbers**

Eight mice were infected with 300 larvae of *T. spiralis*. One week later, these animals together with the same number of control mice were injected with 10 cysts of *T. gondii* intraperitoneally. After another 4 weeks, the numbers of brain cysts were counted. There were no significant differences between the two groups: 1450 ± 250 S.E.M. cysts were recovered from mice infected with *T. spiralis* and 1000 ± 130 S.E.M. cysts were obtained from control animals.

**DISCUSSION**

Concurrent infection with *T. gondii* produced a number of alterations in the host–parasite relationship in murine trichinosis. The most striking of these were persistence of adult worms in the gut, smaller numbers of muscle larvae after intravenous injection of newborn larvae, reduction of inflammation around *T. spiralis* cysts in the muscles and suppression of eosinophilia.

Adult worms are expelled spontaneously approximately 2 weeks after first exposure to *T. spiralis*. This is presumably due to an inflammatory reaction initiated by a variety of mechanical, chemical, enzymatic and antigenic stimuli (Larsh & Race, 1975; Castro, 1976). The elimination in mice concurrently infected with *T. gondii* was delayed approx 1 week. The same phenomenon has been observed when animals have been exposed to *Corynebacterium parvum* (Ruitenberg & Steerenberg, 1973), B.C.G., attenuated *Mycobacterium bovis* (Grove & Civil, 1978) and *Listeria monocytogenes* (Franco, Butler & Grove, unpublished observations).

The mechanisms by which these agents inhibit the expulsion of worms is obscure. They are all immunostimulants and activate macrophages, but under certain conditions of dose, timing and route of administration, may act as immunosuppressants. The immunosuppressive effects of *T. gondii* in-
fection are well described. Infected mice have thymic atrophy (Huldt et al., 1973), depressed levels of haemaggutibilins, haemolysins and spleen plaque forming cells after immunisation with sheep red cells (Strickland et al., 1973; Strickland & Sayles, 1977) and suppression of spleen cell responsiveness to the T cell mitogens, phytohaemagglutinin and concanavalin A, and to the B cell mitogen, lipopolysaccharide (Strickland et al., 1975). T. gondii may inhibit worm expulsion by this immunosuppressive action. Alternatively, it may alter lymphocyte traffic through the gut as has been described with B.C.G. (Zatz, 1976), or act in some other as yet undetermined way which creates a more favourable intestinal milieu and allows prolonged retention of adult worms. This effect is seen, however, only in acute toxoplasmosis, for when animals infected with T. gondii for 4 months were tested, no delay in expulsion was observed.

Many factors determine the number of infective larvae which can be isolated from the muscles. These include the number and fecundity of female worms, the length of time which they persist in the gut, and the largely unknown factors which influence the migration of newborn larvae through the gut and bloodstream, their penetration into muscles and their maturation into infective larvae. Since the adult worms which persisted in the gut of mice infected with T. gondii were fecund, the greater total production of newborn larvae should have been reflected in increased numbers of muscle larvae. There were, in fact, no significant differences between the two groups, thus suggesting that either the systemic migration of these larvae or their maturation in the muscles was impaired in mice infected with T. gondii.

This was confirmed when the effects of T. gondii on the systemic phase of Trichinella infection were studied in isolation with bypassing of the intestinal phase by direct injection of newborn larvae into the veins. Significantly less infective larvae were subsequently recovered from the muscles of mice infected with T. gondii. It has been shown that an intact cellular immune system (Walls, Carter, Leuchars & Davies, 1973; Gore, Burger & Sadun, 1974) and eosinophils (Grove et al., 1977) are important factors in resistance to the systemic phase of Trichinella infection. The suppression of cell-mediated immunity which has been shown by others in toxoplasmosis (Hibbs et al., 1973; Huldt et al., 1973; Strickland et al., 1975) might therefore be expected to lead to increased numbers of muscle larvae. The impaired eosinophilic response to T. spiralis in mice infected with T. gondii may result from immunosuppression since Basten & Beeson (1970) have shown that the development of an eosinophilia depends upon an intact cell-mediated immune system. The failure to develop an eosinophilic response should also facilitate the development of muscle larvae. The observed heightening of
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