Trichinella spiralis: Effects on the Host–Parasite Relationship in Mice of BCG (Attenuated Mycobacterium bovis)

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GROVE, D. I., AND CIVIL, R. H. 1978. Trichinella spiralis: Effects on the host–parasite relationship in mice of BCG (attenuated Mycobacterium bovis). Experimental Parasitology 44, 181-189. The effects of BCG (Bacillus Calmette-Guérin, i.e., attenuated Mycobacterium bovis) on the host–parasite relationship in murine trichinosis were examined. A total of 2 × 10⁴ colony forming units of BCG given iv 1 week prior to Trichinella spiralis infection delayed the expulsion of adult worms from the gut. The suppression of adult worm elimination was proportional to the dose of BCG given. This finding was associated with a reduction in the degree of partial villous atrophy induced in the small bowel by T. spiralis. Adult female worms were fecund when they were examined 1, 2, and 3 weeks after infection of mice with T. spiralis. Despite the prolongation of fecund adult worms in the gut, there were no significant differences in muscle larval counts 4 and 6 weeks after infection. When newborn larvae were cultivated in vitro and injected iv, there was a significant 25% reduction in larval numbers recovered from the muscles of BCG-treated mice 4 weeks later. The administration of BCG had no effect on the inflammatory reaction around larvae in the muscles 4 and 6 weeks after infection. It is concluded that BCG alters the host–parasite relationship producing retention of adult worms in the gut, reduction in the severity of partial villous atrophy, and increased nonspecific resistance to the systemic larval phase of this parasite.

INDEX DESCRIPTORS: Trichinella spiralis; Nematode; Adult worms; Newborn larvae; Trichinosis; Mouse; Partial villous atrophy; Nonspecific resistance; BCG – Bacillus Calmette-Guérin = attenuated Mycobacterium bovis.

INTRODUCTION

Administration of the attenuated strain of Mycobacterium bovis, Bacillus Calmette-Guérin (BCG), stimulates nonspecific resistance in a wide variety of neoplastic (lead articles, British Medical Journal 1976, Lancet 1976) and infectious diseases. Evidence of protection may be seen in a number of protozoal infections such as those of Babesia species, Plasmodium species (Clark et al. 1976), Leishmania species (Smrkovski and Larson 1977, Weintraub and Weinbaum 1977), and Trypanosoma cruzi (Kuhn et al. 1975). Injection of living BCG also induces a high degree of resistance to multicellular helminths. BCG suppressed both the growth and metastasis of Echinococcus multilocularis (Rau and Tanner 1975) and inhibited the development of secondary cysts of E. granulosus (Thompson 1976). Furthermore, BCG reduced both the number of schistosomula recovered from the lungs and the number of adult worms of Schistosoma mansoni in
the portal veins (Fauve and Dodin 1976; R. H. Civil, K. S. Warren, and A. A. F. Mahmoud, in press).

When the effect of another immuno-stimulant, Corynebacterium parvum, was investigated in rats infected with Trichinella spiralis, it was observed that the expulsion of adult worms from the gut was delayed (Ruitenberg and Steerenberg 1973). These observations suggested that BCG may alter the host–parasite relationship in trichinosis. Infection with T. spiralis offers the opportunity to observe the effects of BCG on several stages of the parasite life cycle such as the fecundity of female worms, expulsion of adult worms from the gut, migration of newborn larvae through the bloodstream, and the development of infective larvae in the muscles. Furthermore, other indices of the host response to the parasite may be determined by quantifying partial villous atrophy in the small intestine and the degree of inflammation around organisms in the muscles. We have therefore examined these parameters in mice injected with BCG then infected with T. spiralis.

**Materials and Methods**

**Mice.** Outbred female Swiss albino mice (CF-1), 18 to 20 g in weight, were obtained from Carworth Farms, Inc. (New City, New York, U.S.A.).

**BCG.** The BCG strain of Mycobacterium bovis was obtained from the Research Foundation (Chicago, Ill., U.S.A.). Each ampoule of lyophilized BCG was reconstituted with distilled water and diluted to the appropriate concentration with 0.15 M (physiological) saline; then 0.4 ml was injected iv.

**Nematode infection.** A colony of infected mice was established from a strain of Trichinella spiralis originally supplied by Dr. W. C. Campbell (Merck Institute for Medical Research, New Jersey, U.S.A.). Larvae were isolated by maceration of carcasses in a Waring Blendor, incubation in 1% acid-pepsin for 2 to 3 hr at 37 C, sedimentation in saline, and filtration through gauze (Gould 1970). Mice were infected with 300 larvac in 0.2 ml of saline by esophageal intubation with a 16-gauge needle.

**Intestinal worm burdens.** Adult worms, i.e., nematodes, 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- to 3-cm pieces, and digested in 0.01 M NaOH in 100 ml of 0.15 M saline overnight at 4 C. The intestinal fragments were shaken vigorously and poured into a petri dish, the pieces were removed, and the fluid was transferred to 50-ml centrifuge tubes. The worms were spun down at 400g for 15 min and the total number in the sediment was counted.

**Muscle worm burdens.** The number of larval nematodes in the musculature of each carcass was determined. Every animal was decapitated, skinned, eviscerated, macerated, and digested for 4 hr 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 was counted in duplicate.

**Newborn larvae.** Newborn larvae of T. spiralis were obtained using a modification of the method of Dennis et al. (1970). Thirty mice were each infected with 800 larvae by esophageal intubation. Seven days later, the small intestines were removed, slit longitudinally, and layered on coarse wire mesh racks in beakers containing 0.15 M saline. After incubation for 2 to 3 hr at 37 C, the sediment was collected, and the adult worms were washed several times in 0.15 M saline by sedimentation. They were finally suspended in the following medium: RPMI-1640 with glutamine (Grand Island Biological Company, Grand Island, New York, U.S.A.) (70% by volume), decomplemented fetal calf serum (KC Biological Inc., Lenexa, Kansas, U.S.A.) (29% by volume), and penicillin-
streptomycin 5000 IU/ml and 5000 µg/ml (Flow Laboratories, Rockville, Maryland, U.S.A.) (1% by volume). Aliquots of 15 ml of medium containing approximately 100 worms were transferred to 30-ml tissue culture flasks (No. 3012, Falcon Plastics, Los Angeles, California, U.S.A.) and incubated for 36 hr at 35 C. Newborn larvae were separated from adult worms by passage through a sterile 325-gauge wire mesh (Tyler Co., Cleveland, Ohio, U.S.A.), reconstituted to the required concentration in 0.5 ml of medium, then injected into mice via the tail vein. Four weeks later, the numbers of muscle larvae were determined as described earlier, except that in view of the low numbers, multiple 0.2-ml samples were counted for each carcass.

**Fecundity.** Mice were infected with 300 larvae of *T. spiralis.* The small intestines were removed, and adult worms were recovered 1, 2, and 3 weeks after infection as described above. Approximately 50 worms from each mouse were incubated in 15 ml of medium in tissue culture flasks at 35 C for 24 hr. Adult worms and newborn larvae were then centrifuged at 400g for 10 min, and the numbers of female adult worms and newborn larvae were counted.

**Small bowel pathology.** Segments of small intestine 1.5 cm in length were excised 10 cm below the gastroduodenal junction, opened longitudinally, carefully orientated on filter paper, and fixed in 10% formalin. After routine processing, biopsies were stained with hematoxylin and eosin. The villus: crypt ratio of jejunal mucosa was determined by measurement of villus and crypt heights in 10 representative, well-oriented villi in at least two separate portions of each biopsy (Roberts-Thomson et al. 1976).

**Muscle pathology.** The right hamstring muscles were removed, fixed in 10% formalin, sectioned, and stained with hematoxylin and eosin. The areas of inflammation around 10 isolated larvae cut longitudinally were measured with a πMC particle measurement computer system (Millipore Corp., Bedford, Massachusetts, U.S.A.) (Grove and Warren 1976).

**RESULTS**

**Intestinal Worm Burdens**

Forty-five mice were given $2 \times 10^7$ colony forming units (CFU) of BCG iv. One week later, these mice together with the same number of control mice were infected with 300 larvae of *Trichinella spiralis.* Intestinal worm burdens were determined 4, 7, 12, 17, and 28 days later (Fig. 1). There were no significant differences in worm burdens between the two groups at 4 and 7 days. By 12 days after infection, worm expulsion had begun in the control mice as shown by the worm burden falling to 65% of the original value. In mice treated with BCG, however, no change had occurred; the difference between the two groups was statistically significant ($P < 0.05$, t test). Seventeen days after infection, worm expulsion had commenced in mice given BCG, the worm burden being 75% of the original value, but recovery of worms from control mice had fallen further to 30% of the initial level ($P < 0.01$, t test). Four
FIG. 2. The numbers of adult worms recovered from the gut 14 days after infection of mice with 300 larvae of *Trichinella spiralis*. Mice had been given varying doses of BCG iv 1 week before infection. Eight mice were in each group.

weeks after infection, very few worms (4%) were present in control mice, while in mice treated with BCG, 25% of the original number of worms remained, the difference between the two groups still being significant ($P < 0.005$, $t$ test).

The effect of varying amounts of BCG was determined by giving groups of eight mice doses of BCG ranging from $4 \times 10^5$ to $5 \times 10^7$ CFU in serial fivefold dilutions. One week later, these mice plus a group of control mice were infected with 300 larvae of *T. spiralis*, then intestinal worm burdens were measured after a further 14 days. The number of worms recovered from the small intestines was proportional to the dose of BCG given (Fig. 2). Significantly increased numbers of worms were found in mice given $5 \times 10^7$ CFU ($P < 0.005$, $t$ test) and $1 \times 10^7$ CFU ($P < 0.025$, $t$ test) when compared with control mice. Mice receiving lower doses of BCG did not harbor significantly increased numbers of worms.

**Muscle Worm Burdens**

Eighteen mice were given $2 \times 10^7$ CFU of BCG iv. One week later, these mice together with the same number of control mice were infected with 300 larvae of *T. spiralis*. The numbers of muscle larvae were counted 4 and 6 weeks after infection. Less larvae were recovered from BCG-treated mice ($56,000 \pm 6900$ (±SE)) than from control mice ($72,000 \pm 4600$ (±SE)) 4 weeks after infection, but this difference was not statistically significant. Although adult worms persisted in the gut of mice given BCG, no significant differences in muscle larvae were found between the two groups 6 weeks after infection, the numbers recovered being $58,000 \pm 9700$ (±SE) in control mice and $59,000 \pm 5800$ (±SE) in BCC treated animals.

**Fecundity**

Eighteen mice were given $2 \times 10^7$ CFU of BCG iv. One week later, these mice together with the same number of control mice were infected with 300 larvae of *T. spiralis*. One week after infection, adult worms were recovered and their fecundity was measured. There were no significant differences between the two groups: Worms recovered from six control mice produced $34.4 \pm 4.9$ newborn larvae per female worm per 24 hr, while those obtained from six mice treated with BCC gave $35.8 \pm 3.3$ newborn larvae per female worm per 24 hr. When worms were recovered from BCG-treated mice 2 and 3 weeks after infection with *T. spiralis*, they were still fecund; 50.4 newborn larvae per female worm and 29.6 newborn larvae per

<table>
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<th>TABLE I</th>
<th>Recovery of <em>Trichinella spiralis</em> Muscle Larva 4 Weeks after Intravenous Injection of 1250 Newborn Larvae in Normal and BCG-Treated Mice</th>
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$^a$ $t$ test.
female worm were produced at each time point, respectively. When worms were recovered from control mice 2 weeks after infection with *T. spiralis*, they also were fecund, producing 23.5 newborn larvae per female. Fecundity could not be measured 3 weeks after infection of control mice because insufficient numbers of adult worms were recovered.

**Resistance to Newborn Larvae**

Twelve mice were given $2 \times 10^7$ CFU of BCG iv. Two weeks later, these mice together with the same number of control mice were injected with 8500 newborn larvae iv. All the mice given BCG plus newborn larvae died within 24 hr while none of the control mice given newborn larvae died. Histological examination of the lungs removed from one of the mice given BCG and newborn larvae showed gross hemorrhagic pneumonitis.

In a second experiment, 12 mice were given $1 \times 10^7$ CFU of BCG iv. One week later, these mice together with eight control mice were injected with 1250 newborn larvae by the same route. Four weeks later, muscle larval counts were performed. There was a significant 25% reduction in the number of muscle larvae in mice treated with BCG compared with control mice (Table I).

**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 treated with BCG and from control mice when these groups were examined 4 days after infection with 300 larvae of *T. spiralis* ($P < 0.005$, *t* test) (Fig. 3). The mean value ±SE for this ratio in 16 normal, uninfected mice was $3.1 \pm 0.1$. During Days 7 through 17, the degree of partial villous atrophy did not change in mice given BCG, but it became progressively more severe in the control mice given *T. spiralis* only. When the values of Days 12 and 17 were pooled, the difference between the two groups was statistically significant ($P < 0.005$, *t* test). By 28 days after infection, however, the villus:crypt ratio in the mice given *T. spiralis* only was returning toward normal. The mucosa had become less atrophic than in the BCG-treated mice in which the partial villous atrophy remained unchanged ($P < 0.02$, *t* test).

**Muscle Inflammation**

The degree of inflammation around muscle larvae was measured 4 and 6 weeks after infection. No significant differences were found between the two groups. The geometric mean areas and ranges 4 weeks after infection were 2800 (570 to 19,900) and 3480 $\mu m^2$ (520 to 22,900) for control and BCG-treated mice, respectively. The corresponding values 6 weeks after infection were 4000 (710 to 22,400) and 4770 $\mu m^2$ (1170 to 19,500).

**Discussion**

The administration of BCG produced a number of alterations in the host–parasite relationship in murine trichinosis. The most
striking changes were persistence of adult worms in the gut and reduced numbers of muscle larvae after the iv injection of newborn larvae.

The presence of Trichinella spiralis adult worms in the gut is associated with intestinal mucosal inflammation (Larsh and Race 1975). This inflammatory reaction may be initiated by a variety of mechanical, chemical, enzymatic, and antigenic stimuli (Castro 1976). There is infiltration of the intestinal mucosa with white cells and development of a moderately severe partial villous atrophy. This hostile environment presumably leads to expulsion of the adult worms after which there is resolution of the inflammation and restoration of the architecture and physiology to normal (Larsh and Race 1975; Castro 1976).

We have observed two effects of the administration of BCG on the intestinal phase of Trichinella infection. First, the spontaneous elimination of adult worms, which normally occurs about 2 weeks after the first exposure to T. spiralis, was delayed approximately 1 week. A similar phenomenon has been observed in rats treated with the adjuvant, Corynebacterium parvum, then infected with T. spiralis (Ruitenberg and Steerenberg 1973). Furthermore, we were able to show that the degree of suppression of worm elimination was directly proportional to the dose of BCG administered. Second, the administration of BCG reduced the severity of partial villous atrophy induced by T. spiralis infection but delayed the restoration of the mucosal architecture to normal. This latter observation is not surprising as a number of chronic infections have been associated with prolonged partial villous atrophy (Shiner 1976).

It is unclear whether the retention of adult worms results from this amelioration of partial villous atrophy or whether both of these findings are merely epiphenomena. Ruitenberg and Steerenberg (1973), who did not study the pathological changes in the gut, interpreted their results to indicate that C. parvum exerted an immunosuppressive action on T. spiralis infection. While both C. parvum and BCG are usually immunostimulants, under certain conditions of dose, timing, and route of administration they may act as immunosuppressants (Mathe et al. 1973; Mitchell et al. 1973; Doft et al. 1976). It is also possible that they could delay the elimination of adult worms by altering the traffic of lymphocytes through the gut (Zatz 1976). Alternatively, these agents may, in some other as yet undetermined way, create a more favorable milieu which is manifested by a less disturbed intestinal mucosa and prolonged retention of the adult worms.

A large number of factors determine the number of infective larvae which can be isolated from the muscles. These include the fecundity of the female worms, the length of time which they persist in the intestines, the largely unknown elements which influence the migration of newborn larvae through the gut and bloodstream, their penetration into the muscles, and maturation into infective larvae. Prior exposure to T. spiralis, either by infection or by immunization with antigenic fractions, hastens the expulsion of adult worms from the gut (Grove et al. 1977) and reduces their fecundity (Despommier et al. 1977). In contrast, the administration of BCG nonspecifically delays the expulsion of adult worms and has no effect on their fecundity.

Since the adult worms persisting in BCG-treated mice were fecund, the greater production of newborn larvae should have been reflected in increased numbers of muscle larvae. The numbers recovered from BCG-treated and from control mice 6 weeks after infection, however, were the same despite persistence of adult worms. This suggests that the systemic migration of these larvae or their maturation in the
muscles was impaired in mice treated with BCG.

This was confirmed when the effects of BCG on the systemic phase of *Trichinella* infection were studied in isolation by the injection of newborn larvae directly into the veins. When newborn larvae were injected iv in the first experiment, a severe hemorrhagic pneumonitis was produced and all the mice died. This is an artificial situation, however, for large numbers of larvae were injected over an extremely short period. In natural infections, it is probable that much smaller numbers of larvae are passing through the lungs at any given time. Furthermore, the lungs were probably already involved with heavy granulomatous inflammation and were less resistant to this insult (Youmans and Youmans 1964). Since the granulomatous reaction is directly proportional to the dry weight of BCG administered (Youmans and Youmans 1964), mice were given a reduced dose of BCG and a smaller number of newborn larvae were injected after a shorter interval. In this case, no mice died and there were significantly less larvae in the muscles of BCG-treated animals 4 weeks later.

There was no difference in the inflammatory response around larvae in the muscles 4 and 6 weeks after infection and it is difficult to envisage that BCG alters the maturation of larvae once they are within the muscle cells. It seems more likely that BCG exerts its effects on newborn larvae as they migrate through the circulatory system. It is possible that a major site of such impairment is in the lungs which are involved with granulomatous inflammation.

BCG induces hyperplasia of the reticuloendothelial system (Blanden et al. 1969) and activation of macrophages (Ratzan et al. 1972) in mice. Furthermore, this agent increases the magnitude and duration of delayed hypersensitivity reactions (Mackaness et al. 1974), the production and release of non-antibody mediators into the circulation (Younger and Salvin 1973), and the generation of "natural killer cells" (Wolfe et al. 1976). In addition, BCG can increase antibody production under some circumstances (Miller et al. 1973). Nevertheless, the precise mechanism of action of BCG immunization in trichinosis is uncertain. It is unclear whether lung inflammation *per se* is related to the increased resistance to the systemic phase of *T. spiralis*. It has been shown that an intact cellular immune system (Walls et al. 1973; Gore et al. 1974) and eosinophils (Grove et al. 1977) are important factors in resistance to the systemic phase of *Trichinella* infection. While there is no evidence that BCG stimulates eosinophils, the activation of macrophages and stimulation of cytotoxic lymphocytes by BCG could account for the increased protection observed. The location and timing of this effect and the mechanism of such an activity, however, remain to be determined.

Other studies have shown that BCG non-specifically stimulates resistance to a wide variety of infectious agents. This report supports this concept by extending observations to another helminth species. It provides a model in which the host–parasite relationship can be studied. In particular, it illustrates the opportunity to dissect and define the separate effects of BCG on the intestinal and systemic phases of this helminth infection.

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