Immunosuppression and Increased Susceptibility to Japaneses B Encephalitis Virus in *Trichinella spiralis*-Infected Mice (37345)

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The ability of infections involving systemically migrating nematode larvae to enhance the virulence of superimposed viral infections has been reported for several different systems, as recently reviewed by Woodruff (1). This promoting effect has generally been attributed to mechanical traumatization of the target tissues of the virus by the migration of nematodes. However, recent reports indicated that mice infected with *Trichinella spiralis* demonstrated prolonged survival of allografts (2) and decreased antibody response to injected sheep erythrocytes (3). The purpose of this investigation is to examine the response of mice infected with *T. spiralis* to peripheral challenge with a neurotropic viral agent, Japanese B encephalitis (JBE) virus, in light of the above findings. *Nematospiroides dubius*, a gastrointestinal nematode of mice whose life cycle contains no extragastrointestinal stages, was chosen as a control parasitic infection.

Materials and Methods. Female 8- to 9-week-old Swiss albino mice, HaM/ICR, were obtained from Charles River Breeding Laboratories, Wilmington, MA. The preparation of *N. dubius* infective third-stage larvae, and the details of gastric administration have been recently described (4), as have those for *T. spiralis* larvae (5). Nonlethal gamma irradiation of control larvae was performed using a ^{60}Co source at a dose of 22,000 rads in 4 min. JBE virus (Nakayama strain) was prepared as a 20% (w/v) suspension of infected suckling mouse brain, and stored at −70°. The weanling mouse intracerebral neutralization test and micro complement-fixation (CF) test were performed as previously described (6). Survival time index (STI, 1000 times the reciprocal of the harmonic mean survival time) was determined by the method of Gordon Smith and Westgarth (7). Clearance of carbon following iv injection was assessed by the colorimetric method previously reported (8), utilizing Pelikan Ink C-11-1431A (Gunther Wagner, Hanover, Germany) as a source of colloidal carbon. The kinetics of carbon clearance were first order; K represents the absolute value of the slope of the plot of log blood carbon concentration (determined as optical density) vs time. Total serum corticosteroids were determined using the method of Murphy (9).

Results. Two groups of 200 mice each were infected orally with 200 *N. dubius* or *T. spiralis* larvae. Two other groups received in a similar manner 200 irradiated *T. spiralis* or irradiated *N. dubius* larvae. A fifth group received nothing and served as controls. At 7 or 28 days following infection, mice were challenged with JBE virus, 0.3 ml sc, at doses of 10^5–10^{10} suckling mouse intracerebral LD_{50} ($SMICLD_{50}$), 10–30 mice of each group per 10-fold dilution. Mice were observed for mortality for 21 days. At various times after virus challenge, animals receiving 10^8 $SMICLD_{50}$ were sacrificed and blood and brains were harvested. Figure 1 shows the STI of each group at each virus dose.

Mice infected with *T. spiralis* 7 days prior to virus challenge were much more prone to develop viral CNS disease than controls or mice previously given *N. dubius*, irradiated *T. spiralis*, or irradiated *N. dubius* (Fig. 1a). The STI was related to $\log_{10}$ JBE dose for *T. spiralis*-infected animals only. In the *T. spiralis* group, the harmonic mean survival time was about 10 days at the highest JBE doses employed (with nearly 100% mor-

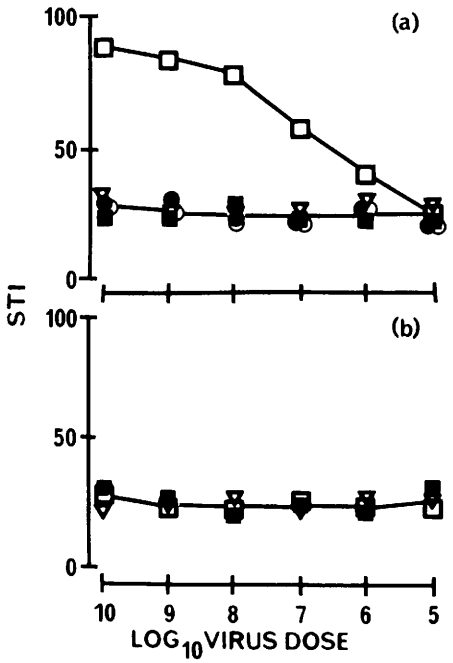

FIG. 1. Effect of previous helminthic infections on survival time indices of mice challenged with JBE. Mice were orally infected with 200 live or non-lethally irradiated larvae. After (a) 7 or (b) 28 days, they received 10^5–10^{10} $SMICLD_{50}$ of JBE sc and observed daily for mortality. Survival time index $(STI) = (10^3/R)\ (\sum_{i=1}^{R} 1/t_i)$, where R is the number of mice per dose, and t_i is the survival time of the ith mouse. Open squares, *T. spiralis*; closed squares, irradiated *T. spiralis*; open circles, *N. dubius*; closed circles, irradiated *N. dubius*; triangles, controls.

tality). At lower doses, survival time became longer and mortality lower, until the control situation was approached at the lowest dose of virus (10^5). In all other groups, death was rare, occurring randomly at all virus doses, and the group harmonic mean survival time among those few which died was about 15 days. If virus was given at 28 days following administration of normal or irradiated *T. spiralis* larvae (Fig. 1b), mortality, STI, and survival time were similar to those of controls. *N. dubius*-infected mice were not examined for susceptibility to JBE virus at this time.

Examination of the carcasses of mice at 7, 14, and 28 days after administration of larvae demonstrated the following points: (a) Neither *N. dubius* nor *T. spiralis* larvae could be detected microscopically in squashed brain preparations of infected mice. (b) Brains of either *N. dubius*- or *T. spiralis*-infected mice did not show any gross evidence of pathology in the form of hemorrhages, petichiae, enlargement, or edema. (c) Worm burdens for both parasites at 28 days following oral infection were similar to previously published findings (4, 10) using these same helminth dosages. (d) Neither *T. spiralis* encysted larvae or adult *N. dubius* forms were found in mice receiving irradiated larvae.

Two experiments were performed in an effort to elucidate the mechanism of increased susceptibility to JBE in *T. spiralis*-infected mice. At 7 and 21 days following helminthic infection, two mice from each group, not given virus, were exsanguinated and pooled serum was assayed for total corticosteroid levels. As noted in Table I, both *T. spiralis* and *N. dubius* infection elevated serum corticosteroid concentration to the same level by the 7th day. Therefore, it appears that changes in endogenous corticosteroid levels following *T. spiralis* infection do not appear to be responsible for the increased susceptibility to JBE virus in *T. spiralis*-infected mice. Farber and Glasgow (11), using pregnant and lactating rats, also found no correlation between endogenous corticosteroid levels and susceptibility to encephalomyocarditis virus.

Another set of mice, not given virus, were examined at 7, 14, 28, and 49 days (6 mice

TABLE I. Effect of Nematode Infection on Serum Corticosteroid Levels at 7 and 21 Days Following Infection.[a]

Group	Total corticosteroids (μg/100 ml) at 7 days	21 days
Normal *T. spiralis*	31.3	23.1
Irradiated *T. spiralis*	22.7	22.3
Normal *N. dubius*	31.2	23.7
Irradiated *N. dubius*	19.6	21.3
Control	21.6	24.2

[a] No virus challenge given.

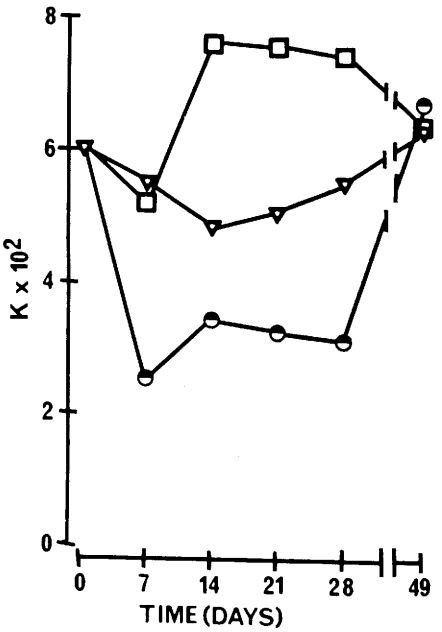

FIG. 2. Effect of helminthic infection on the rate of carbon clearance (K) from blood following intravenous injection at various times after infection. Rate constant K is calculated at the slope of straight lines generated from plot of log (OD) vs time at which blood samples taken after injection of carbon (not shown). Squares, *T. spiralis*; circles, *N. dubius*; triangles, controls.

per group per time) for fixed reticuloendothelial (RE) activity following intravenous injection of colloidal carbon. Figure 2 shows that the average rate of clearance of carbon particles was increased significantly from Day 14 to 28 following *T. spiralis* infection. In contrast, infection with *N. dubius* depressed carbon clearance from Day 7 to 28. By 49 days, the carbon clearance rate returned to control values for mice infected with either helminth. It is concluded that the changes in the phagocytic activity of the fixed RE cells, as measured by carbon clearance do not explain the increased susceptibility of *T. spiralis*-infected mice to JBE virus.

Pools of sera were obtained from the various groups of mice 14 days following JBE virus challenge. Tables II and III show that the humoral CF and neutralizing antibody responses to the virus in *T. spiralis*-infected mice were significantly lower than in

TABLE II. Effect of Nematode Infection on Development of CF Antibody 14 Days after Challenge with 10^8 $SMICLD_{50}$ of Virus.

Group	Serum CF antibody titer at[a]	
	21 days[b]	42 days[b]
Normal *T. spiralis*	4	4
Irradiated *T. spiralis*	64	32
Normal *N. dubius*	16	ND[c]
Irradiated *N. dubius*	16	ND
Control	32	32

[a] CF test performed by a modification of the Fulton–Dumbell technique, as described previously (6). Four dilutions of sucrose acetone-extracted antigens were tested against seven serum dilutions, beginning at 1:4. Results above are reciprocals of serum dilution endpoints at an antigen dilution of 1:16. Antigen dilutions of 1:8 and 1:32 gave virtually identical serum titers. Titers shown are geometric means of results on pools of 6 mice each.

[b] Time interval between helminthic infection and exsanguination.

[c] Not done.

controls, or in mice challenged with virus after receiving *N. dubius*, irradiated *N. dubius*, or irradiated *T. spiralis* larvae. (Mice from all groups were negative ($<$ 1:4) for viral CF and neutralizing antibody on the day of virus challenge.) The results indicate that infection with *T. spiralis* larvae which were capable of normal development suppressed the measured humoral immune response to

TABLE III. Effect of Nematode Infection on Development of Neutralizing Antibody 14 Days after Challenge with 10^8 $SMICLD_{50}$ of Virus.

Group	Log_{10} serum neutralizing index[a] at	
	21 days	42 days
Normal *T. spiralis*	1.44	1.13
Irradiated *T. spiralis*	2.59	2.33
Normal *N. dubius*	2.72	ND
Irradiated *N. dubius*	2.39	ND
Control	2.39	2.67

[a] Determined as the difference between the log_{10} LD_{50} of JBE in the presence of normal mouse serum and test serum, by the method of Reed and Muench (12).

JBE virus. Since this immunosuppression was observed at both 7 and 28 days following administration of *T. spiralis*, and these mice were only more sensitive to virus at 7 days, it is considered unlikely that the suppression of humoral immunity *per se* increased susceptibility to virus at 7 days.

To elucidate further the humoral immunosuppression found in *T. spiralis*-infected mice, the experiment was repeated, with the *T. spiralis* and control groups only. Two mice per group were exsanguinated at various times after virus infection. Sera from each two mice were pooled. Results in Table IV show that on Day 8 the CF antibody response to virus in Trichinella-infected and control mice was similar, but by Day 12, significant suppression was evident in the former group. This occurred whether the virus challenge was given 7 days or 28 days after the *T. spiralis* larvae were administered.

Discussion. Oral infection of mature mice with 200 *T. spiralis* larvae 7 days prior to peripheral JBE virus challenge greatly increased their susceptibility to fatal viral CNS disease. Mice infected with 200 *N. dubius* larvae, or irradiated *T. spiralis* or irradiated *N. dubius* larvae, manifested the same low susceptibility to virus challenge as controls. The increase in JBE mortality and decrease in survival time was a function of virus dose employed in the *T. spiralis* group, whereas, in all other groups, the susceptibility was low and random, and survival time was relatively constant, apparently independent of amounts of virus in the dose range employed. In a similar manner, Kilham and Oliver had shown that *T. spiralis*-infected rats showed increased susceptibility and decreased survival time when challenged with encephalomyocarditis virus (13).

Infection with *T. spiralis* may provide new routes of access for JBE into the central nervous system, since larval migration has been reported in the brains of mice infected with unknown numbers of *T. spiralis* larvae (14). Although no evidence of larval migration or tissue damage could be detected in the brain in our mice, smaller microscopic breaks in the blood–brain barrier might have escaped detection by the gross methods employed here. Also, larvae may have invaded the spinal cord or large nerve trunks during migration.

T. spiralis significantly reduced the production of CF and neutralizing antibody to JBE. This supports previous reports by Faubert and Tanner (3) of suppression of antibody formation against sheep erythrocytes in *T. spiralis*-infected mice. The mechanism of this suppression is not known; however, it required at least some morphogenesis of *T. spiralis* larvae, since nonlethally irradiated worms had no effect on subsequent JBE antibody production. Antigenic competition, as suggested by Adler (15), or a transferable immunosuppressive substance found in the serum of *T. spiralis*-infected mice (3) may explain the immunosuppression seen here.

While Vollmer and Hurlbut (16) showed that administration of large doses of corticosteroids increased the susceptibility of adult mice to JBE virus, the modest increases above control values found by us at 7 days following *T. spiralis* infection are not likely to explain the phenomenon. In addition, *N. dubius* infection resulted in similar elevation of corticosteroids without increasing suscepti-

TABLE IV. Effect of *T. spiralis* Infection on the Development of Viral CF Antibody in Mice Challenged with 10^8 $SMICLD_{50}$ of JBE Virus at 7 and 28 Days after Helminth Infection.

Time interval[a]	Group	CF titer at time (days) after JBE challenge							
		3	4	5	6	8	10	12	14
7	*T. spiralis*	ND	ND	ND	<4	4	4	8	8
7	Control	ND	ND	ND	<4	8	16	32	64
28	*T. spiralis*	<4	<4	<4	<4	8	8	16	16
28	Control	<4	<4	<4	<4	8	16	128	128

[a] Time elapsed between helminthic and viral infections.

bility to JBE virus.

At 7 days after infection with *T. spiralis*, the rate of carbon clearance was unaffected, but was increased at 28 days. Since *N. dubius*-infected mice demonstrated depressed rates of carbon clearance with no increased susceptibility to virus, fixed RE cells probably played little or no role in resistance by clearance of JBE virus. It is, of course, possible that phagocytosis of colloidal carbon and JBE virus are performed by different cells, but there are no data to support this.

Summary. Infection with *T. spiralis* larvae greatly increased the susceptibility of mature mice to fatal CNS disease when challenged peripherally with JBE virus 7 days after administration of larvae. By 28 days following *T. spiralis* infection, susceptibility to JBE virus returned to control levels. It was found that this parasite caused a suppression of the neutralizing and complement-fixing antibody responses to JBE virus, whether challenged at 7 or 28 days. In contrast, infection with *N. dubius* larvae, or irradiated larvae of either parasite, had no effect on susceptibility or antibody responses to virus. Evidence of *T. spiralis* larval migration into the brains of infected mice was not observed. Increased corticosteroid hormone levels, suppression of the humoral antibody response, or changes in fixed macrophage phagocytic activity did not appear to constitute potential mechanisms for the increased susceptibility of *T. spiralis*-infected mice to JBE virus.[1]

[1] After submission of this manuscript, we discovered that work similar to part of that reported here had been previously performed by Col. Bryce Walton, then of Gorgas Memorial Laboratory, Panama. This unpublished work is abstracted in *Proc. First Int. Congr. Parasitol.*, p. 97 (1964) and describes the increased mortality and decreased survival time in *T. spiralis*-infected mice following peripheral challenge with JBE, Ilheus, and eastern equine encephalitis viruses, compared to non-parasitized control mice.

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