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Immunological Sequelae of *Trichinella spiralis* Infection in Mice: Effect on the Antibody Responses to Sheep Erythrocytes and Japanese B Encephalitis Virus

A. S. LUBINIECKI[1]* AND R. H. CYPESS

Department of Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania 15261

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Immunosuppression of the antibody response to Japanese B encephalitis (JBE) virus and sheep erythrocytes (SRBC) was observed in mice infected with *Trichinella spiralis*. This suppression was paralleled by the presence of fewer antibody-forming cells to SRBC in the spleens of parasitized mice. Both primary and secondary complement-fixing antibody responses to JBE virus were suppressed, but the development of immunological memory was not affected. Parasitized mice demonstrated a normal blastogenic response to phytohemagglutinin and normal serum clearance rate of injected ^{125}I-labeled immunoglobulin G_{2b}, although the size of the extravascular fluid compartment was significantly increased. The data presented here and in previous reports suggest that sequential antigenic competition is a possible explanation for the humoral immunosuppression to heterologous antigens caused by *T. spiralis* infection.

Infection of mice with *Trichinella spiralis* results in marked alterations in both the anatomical and functional status of the immune system. The anatomical changes include the distribution, amount, and proportion of histocytes and lymphoblasts in the thymus and a hyperplasia of the spleen and mesenteric lymph nodes (J. A. Molinari, R. H. Cypess, and B. Appel, Int. Arch. Allergy Appl. Immunol., in press). These anatomical changes were accompanied by functional changes in both the cellular and humoral systems. These include increased resistance to intraperitoneal or intravenous challenge with *Listeria monocytogenes* (4, 7), prolonged homograft survival (21), lower incidence of spontaneous mammary carcinoma (24), accelerated clearance of colloidal carbon (3), potentiation in the delayed hypersensitivity skin response to old tuberculin after infection with BCG (5, 17) and a depression in the antibody response to sheep erythrocytes (SRBC) (9) and Japanese B encephalitis (JBE) virus (3, 14). In view of the potential importance of these parasite-induced effects, it was considered of interest to examine more closely the qualitative and quantitative aspects of the antibody response in parasitized animals.

MATERIALS AND METHODS

Parasitic infection. Outbred albino Swiss Webster female mice, 8 to 9 weeks of age, were obtained from Charles River Breeding Laboratories, Wilmington, Mass. *T. spiralis* larvae were prepared from infected muscle as previously described (12). Mice were infected orally with 200 larvae. No observable illness resulted, and this number of infective larvae produce a whole-body worm burden of about 8,000 larvae by day 28 (7).

Hemagglutination (HA) test. One set of mice were given 10^7 SRBC (PBL, Cockeysville, Md.) by the intraperitoneal (i.p.) or intravenous (i.v.) route at various intervals after *T. spiralis* infection. At various time intervals mice were bled from the retro-orbital venous plexus. Individual blood samples were diluted 1:5 in HA buffer (pH 7.3, BBL) containing 10 USP units of heparin per ml. After centrifuging to remove blood cells, plasma was frozen at −70 C until testing. HA tests were performed in microplates (Cooke Engineering Co., Alexandria, Va.) by combining 25 μl of diluted plasma and 75 μl of 0.2% (vol/vol) SRBC in HA buffer supplemented with 0.1 mg of bovine serum albumin per ml to facilitate settling. After incubation at 37 C for 60 min, SRBC were allowed to settle at 25 C for 3 h and then scored for HA.

Plaque-forming cell (PFC) assays. Mice given 10^7 SRBC 14 days after *T. spiralis* infection were sacrificed 3 or 8 days later. The direct assay was performed as described previously (16). The indirect assay was performed as described by Dresser and Greaves (8). Rabbit anti-mouse immunoglobulin G (IgG) was obtained from Meloy Laboratories, Inc., Springfield, Va.

PHA transformation assay. Another set of infected and control mice (five mice per group) was sacrificed on days 7, 12, and 20. Spleens were aseptically removed, rinsed in Hanks balanced salt solution,

[1] Present address: Meloy Laboratories, Inc., Springfield, Va. 22151.

and teased through a no. 60 mesh sieve into 10 ml of RPMI 1640 medium supplemented with 5% newborn calf serum, antibiotics, and 0.2 mg of $NaHCO_3$ per ml. Cell concentrations were adjusted to 8×10^6 mononuclear cells/2 ml, and 2-ml aliquots in duplicate were exposed to various concentration of reagent grade phytohemagglutinin (PHA; Wellcome Laboratories, Beckenham, England). Cultures were maintained at 37 C in a humidified 5% CO_2 atmosphere for 2 days. At this time, cultures were labeled by exposure to [5-^{3}H]thymidine (2 μCi, 6.7 Ci/mmol, New England Nuclear Corp., Boston, Mass.) for 4 h. Cells were then trichloroacetic acid precipitated, washed in methanol, dissolved in hyamine hydroxide, and counted by liquid scintillation counting techniques.

Labeled IgG. A third group of infected and control mice was injected i.v. with ^{125}I-labeled mouse IgG_{2b} at 18 days after infection. Purified mouse IgG_{2b} (Meloy Laboratories) was labeled in the following manner. A solution of 0.25 ml of immunoglobulin (0.85 mg of protein/ml), 0.50 ml of 2× phosphate-buffered saline (pH 7.4), 0.15 ml of carrier-free ^{125}I (1 mCi; ICN, Irvine, Calif.), and 0.1 ml of lactoperoxidase (10 μg/ml; Calbiochem, San Diego, Calif.) was prepared. The reaction was initiated by the addition of 10 μl of 8.8 mM H_2O_2. After 15 min at 25 C, the reaction mixture was chromatographed on a Sephadex G-25 column equilibrated with phosphate-buffered saline at 25 C. The descent of the labeled IgG was monitored by a Geiger counter. The void volume was collected and dialyzed overnight at 4 C against phosphate-buffered saline. Over 99% of the radioactivity recovered after dialysis was trichloroacetic acid precipitable, and the preparation had specific activity of 5.7×10^5 counts/min per μg of protein. At various times after injection with 2.42×10^5 counts/min of ^{125}I-labeled IgG_{2b} (about 0.4 μg of protein in 0.1 ml of phosphate-buffered saline), mice were bled from the retro-orbital venous plexus. Individual blood samples (25 μl) were digested in 0.25 ml of 1.0 N NaOH, neutralized with 0.25 ml of 1.0 N HCl, and counted by liquid scintillation counting techniques (14).

Virus infection. The preparation of JBE (Pekin strain) virus stocks has been detailed elsewhere (13). Primary immunization of mice was accomplished by the subcutaneous injection of 10^8 suckling mouse mean lethal doses. Illness or death rarely resulted (<5%). Secondary immunization consisted of subcutaneous inoculation of formalin-inactivated virus (10) equivalent to 10^{10} suckling mouse mean lethal doses.

Virus serology. Groups of 5 to 20 mice were exsanguinated and the pooled sera frozen at −70 C until testing. Complement fixation (CF) tests were performed in duplicate by previously described methods (11). Fourfold differences between the CF titers of two sera were considered to be statistically significant. Duplicate tests never differed by more than one dilution (twofold). Although the use of pooled sera is less desirable than individual sera, the latter method rapidly becomes impractical as the number of experimental groups increase. Preliminary experiments also showed that the geometric mean CF titers of three groups of 15 sera each were virtually identical to those of the three pooled sera.

RESULTS

HA antibody activity. Mice were examined at various times after *T. spiralis* infection for their ability to produce HA antibody to SRBC. Table 1 shows that infected mice given SRBC at 18 days by the i.v. route produced essentially normal levels of HA antibody, of both 2-mercaptoethanol-sensitive and -resistant types. However, mice given SRBC by the i.p. route at this time produced significantly less 2-mercaptoethanol-resistant HA antibody ($P < 0.005$ by Student's *t* test at 7, 14, and 28 days after SRBC administration) but normal levels of 2-mercaptoethanol-sensitive HA antibody at 7 days. HA antibody activity in all groups examined at 14 and 28 days was 2-mercaptoethanol resistant. Administration of SRBC by the i.p. route at 56 or 72 days after *T. spiralis* infection resulted in normal levels of 2-mercaptoethanol-resistant HA antibody. Thus, the activity of circulating

TABLE 1. *Effect of T. spiralis infection on HA antibody response to i.v.- or i.p.-administered SRBC*

Time (days)[a]	Route	Group[b]	HA titer[c] on day:			2-ME HA titer[d] on day:		
			7	14	28	7	14	28
18	i.v.	Con	151	94.5	69.8	13.9	86.1	83.0
18	i.v.	Ts	123	53.6	58.0	10.5	58.2	86.1
18	i.p.	Con	186	260	128	19.3	225	128
18	i.p.	Ts	142	24.6	58.0	6.5	28.0	70.7
56	i.p.	Con	ND[e]	242	ND	ND	219	ND
56	i.p.	Ts	ND	265	ND	ND	205	ND
72	i.p.	Con	ND	291	ND	ND	260	ND
72	i.p.	Ts	ND	284	ND	ND	248	ND

[a] Interval between *T. spiralis* infection and SRBC administration by indicated route.
[b] Con, Control mice; Ts, *T. spiralis*-infected mice.
[c] Reciprocal of highest dilution showing positive HA (geometric mean of 15 individual samples) at time shown after SRBC administration.
[d] HA titer after exposure to 100 mM 2-mercaptoethanol (2-ME), 37 C for 60 min.
[e] ND, not done.

HA antibody, putatively of the IgG class, was reduced by prior *T. spiralis* infection only if the i.p. route was employed and the SRBC were administered before day 56 postinfection.

PFC assay. *T. spiralis*-infected and control mice were given 10^7 SRBC at 14 days and sacrificed 3 or 8 days later. Table 2 shows that the numbers of direct and indirect PFC were not significantly affected by *T. spiralis* infection when the SRBC were inoculated i.v. In contrast, i.p. injection of SRBC resulted in significant reduction in the direct and indirect PFC values in injected mice.

PHA responses. To test whether *T. spiralis* infection resulted in alterations in the ability of splenic T cells to undergo blast transformation, the PHA response of mononuclear spleen cells was examined as described above. No significant differences were observed between *T. spiralis*-infected and control mice at the time intervals tested (Fig. 1).

Immunoglobulin clearance. As shown in Fig. 2, the curve for the disappearance of ^{125}I-labeled IgG in infected mice is generally lower than the curve for normal mice, but similar in shape. The initial rapid decline in serum radioactivity of both groups was probably due to equilibration with an extravascular compartment (14). Linear regression analysis of the 3-, 6-, and 11-day data for the two groups demonstrated several points. First, the slopes of the regression lines for the infected and control groups are equal and correspond to half-lives of 3.95 and 4.30 days, respectively, for the ^{125}I-labeled mouse IgG_{2b}. Second, the two lines are parallel and noncoincident; their noncoincidence implies that the extravascular compartment was somewhat larger in *T. spiralis*-infected mice than in control mice. By analysis of the intercepts of the regression lines, the extravascular compartment of infected mice effectively removed 67.3% of the inoculum from the serum when at equilibrium, whereas in control mice only 57.3% was removed. This 18% increase in size of the extravascular compartment of *T. spiralis*-infected mice was statistically significant ($P < 0.001$, by Student's t test). Thus, *T. spiralis* infection probably resulted in a somewhat larger extravascular compartment, but did not effect the clearance per se of IgG from the serum.

Antibody to JBE virus. Mice given primary JBE injection at 14 days after *T. spiralis* demonstrated a lower-titered primary CF response than control mice (Fig. 3). When given a second JBE injection on day 28, 42, or 56, the secondary response was normal at the last two time periods tested, but not at the first. Replicate experiments reproduced these results on both occasions. Another experiment (Table 3) demonstrated that in mice given primary JBE challenge at various intervals after *T. spiralis* infection, the immunosuppressive effect was only observed in mice given the virus on or before day 28. It is concluded from these experiments that mice given JBE within 28 days after infection with *T. spiralis* demonstrated a suppression in both the primary and secondary antibody response, but this suppression was not evident if either the primary or secondary immunization was administered after 42 days.

Reports by other investigators (2, 25) and

TABLE 2. *Effect of T. spiralis infection on the development of spleen PFC in response to i.p. or i.v. inoculation of 10^7 SRBC 14 days after infection*

Group[a]	Route	Time[b]	PFC × 10^{-3}/spleen[c]		P value[d]	
			Direct	Indirect	Direct	Indirect
Con	i.v.	3	2.04	ND	>0.10	
Ts	i.v.	3	1.50	ND		
Con	i.p.	3	9.32	ND	<0.01	
Ts	i.p.	3	1.27	ND		
Con	i.v.	8	31.9	3.62		
Ts	i.v.	8	19.4	1.23	>0.10	>0.10
Con	i.p.	8	35.8	14.5		
Ts	i.p.	8	12.0	<0.05[e]	<0.01	≪0.01

[a] Con, Control mice; Ts, *T. spiralis*-infected mice.

[b] Interval between SRBC inoculation and sacrifice.

[c] Geometric mean PFC per spleen, duplicate determinations on eight mice per group. ND, Not done.

[d] Probability that samples for *T. spiralis*-infected and control mice belong to the same population, by Wilcoxon's rank test for two samples, 2-tailed. *P* values were identical if PFC/10^6 viable spleen cells were employed in place of PFC/spleen values shown in this table.

[e] No plaques were detected above direct levels; the value 0.05 would have been equivalent to 1 plaque per 2 dishes.

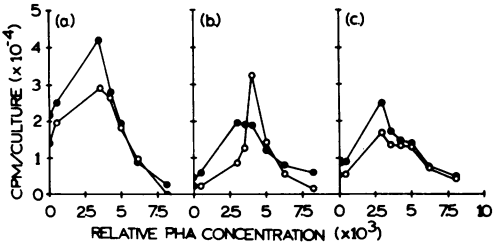

FIG. 1. *Effect of T. spiralis infection on the ability of mouse spleen cells to incorporate [³H]thymidine after exposure to PHA. Cultures were prepared, labeled, and harvested as described in text. Relative concentration refers to the reciprocal of the final dilution factor of PHA present in the culture compared to the undiluted PHA stock reconstituted to the recommended volume (5.0 ml). The dilutions tested were 1:2,000, 1:320, 1:280, 1:240, 1:200, 1:160, 1:120, and 1:20. The last concentration mentioned was uniformly toxic and led to no incorporation above background, and it is not shown. A control receiving only sterile water was included in each set. The 1:320 group was not tested at 7 days. Spleen cultures were established at (a) 7, (b) 12, or (c) 21 days after T. spiralis infection. Open circles represent control mice; closed circles, T. spiralis-infected mice.*

unpublished data from our laboratory indicate that the CF antibody activity to JBE virus is confined exclusively to molecules of the IgG class. Preliminary experiments employing indirect immunofluorescence methods (20) have also failed to detect significant differences in the anti-JBE IgM response between normal and *T. spiralis*-infected mice. It is suggested that *T. spiralis* infection produces a similar type of immunosuppression to both SRBC and JBE virus in that IgG is more inhibited than IgM activity.

DISCUSSION

In this communication, we report that the humoral immunodepression induced by *T. spiralis* infection is of limited duration and is route dependent, involves both primary and secondary antibody responses apparently without affecting immunological memory per se, and appears to affect serum IgG antibody activity to a greater degree than that of IgM. The decrease in serum antibody activity is accompanied by decreased numbers of antibody-forming cells. Although the PFC numbers appear to be inhibited more than the serum antibody activities, the complex relationship between these two parameters precludes any attempts to correlate them.

It is clear from the data presented here, and from previously reported studies (3, 5, 14), that *T. spiralis* infection results in functional changes in the normal immunological responses to heterologous antigens. The observed humoral immunosuppression has been attributed to a number of causes, including antigenic competition (3, 7), increased rates of immunoglobulin clearance (3), and immunosuppressive substances elaborated by *T. spiralis* (7). The present work allows elimination and/or modification of these hypotheses as follows: (i) the serum clearance of IgG was unaltered in *T. spiralis*-infected mice, suggesting that the observed depression in IgG antibody activity levels was not related to IgG turnover and clearance; (ii) the normal blastogenic response of lymphocytes to PHA and the pronounced route dependence of the immunosuppression suggest the inhibition in anti-SRBC antibody-forming cells and serum antibody levels are probably not due to a generalized defect in T cell numbers or function (however, specific defects in T cell function cannot be ruled out); and (iii) the immunosuppressive factor previously reported to be present in parasitized mice (7), if present, is quite specific in its effect since its activity was heavily dependent upon the route of heterologous antigen administration.

The data presented here and our previous

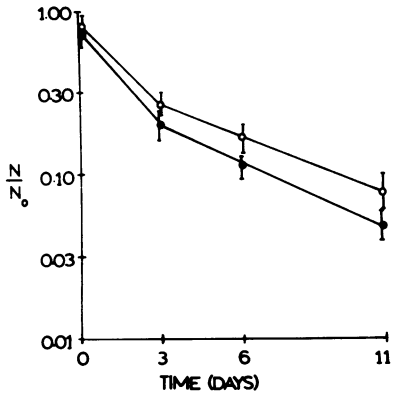

FIG. 2. *Effect of T. spiralis infection on the clearance of i.v. injected ^{125}I-labeled mouse IgG$_{2b}$. Mice were injected 18 days after infection, and sampled at 1 h, 3, 6, and 11 days after inoculation of the labeled immunoglobulin. N_0 was estimated to be 2,700 counts/min per 25 μl blood on the basis, of the inoculum (2.42 × 10⁵ counts/min in 0.1 ml) and the known blood volume of this mouse strain at the age employed here (7.48% of body weight, J. P. Lucas, personal communication). N was obtained for each of 15 mice per group by assessing the radioactivity present in 25 μl of blood. The mean of each group of 15 individual samples is shown, ±1 standard error. Open circles represent control mice; closed circles, T. spiralis-infected mice.*

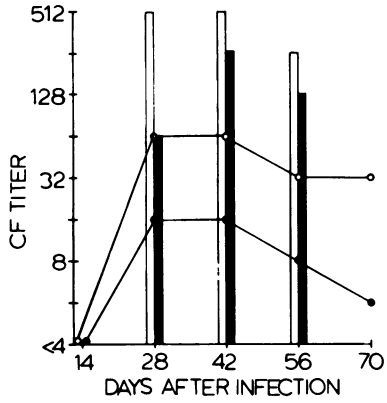

FIG. 3. *Effect of T. spiralis infection on primary and secondary CF antibody responses to JBE virus. All mice received primary immunization on day 14 after T. spiralis infection. The duration of their primary antibody response is designated by circles (open circles, control mice; closed circles, T. spiralis-infected mice). Histograms represent CF antibody titers at 14 days after a second JBE immunization given at the time shown under the histogram (open histograms, control mice; closed histograms, T. spiralis-infected mice). Geometric means of two determinations are presented.*

reports (3, 5, 12) are consistent with the hypothesis of sequential antigenic competition (18, 22). Previous reports on sequential antigen competition involving heterologous erythrocyte systems indicate that they are route and time dependent (1, 10), immunological memory is unaffected (1), and T cell-dependent (or IgG) responses are affected to a much greater degree than B cell-dependent (or IgM) responses (10). Although there have been several suggestions concerning the mechanism responsible for antigenic competition (18), Schrader and Feldman (20) recently described an in vitro system in which the mechanism of antigenic competition appeared to reside in alteration of the surface of macrophages from immunized animals. This modification prevented normal T-B cell interaction and was reversible by addition of normal macrophages to the system or trypsinization of the cultures.

Whether the humoral immunosuppression after *T. spiralis* infection results from alteration of normal macrophage-T-B cell interactions will require further study. However, the observed humoral immunosuppression in parasitized animals has a temporal pattern which parallels increased fixed macrophage phagocytic activity (3) and increased resistance to *L. monocytogenes* (4). Further, no suppression of direct PFC was observed when SRBC were administered i.v. or i.p. at 7 days after *T. spiralis* infection

TABLE 3. *Serum antibody activity 14 days after primary JBE immunization at various intervals following T. spiralis infection*

Time[a]	CF titer[b]	
	Ts	Con
7	8	32
14	16	64
21	8	64
28	8	64
42	32	32
56	32	32

[a] Time interval between *T. spiralis* infection and JBE immunization.

[b] Reciprocal of the highest dilution resulting in ≤50% hemolysis. Con, Control mice; Ts, *T. spiralis*-infected mice.

(15), a time when both carbon clearance and resistance to intraperitoneally administered *L. monocytogenes* was not elevated.

The spleen is the principal organ of antibody formation after i.v. inoculation of particulate antigens in the mouse (16). Thus, the failure to observe marked immunosuppression to SRBC after i.v. inoculation may be due to differences in the macrophage-T-B cell interaction in this organ versus that in the peritoneal cavity. Additional investigations of macrophage, T cell, and B cell function and intercellular cooperation in parasitized animals are presently in progress. In view of the ubiquity of animal parasites and the chronic nature of their infections, their potential to cause a state of altered reactivity of the immune system may have clinical significance, especially in those pathogen-laden environments where the host must maintain strict and constant immunological surveillance to avoid serious disease.

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