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Immunological Sequelae of *Trichinella spiralis* Infection in Mice

II. Potentiation of Cell-Mediated Response to BCG After Infection with *Trichinella spiralis*

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Mice were infected with 200 *Trichinella spiralis* 14 days after intravenous administration of 4×10^6 viable BCG cells. Individual groups were tested for delayed hypersensitive footpad responses at 14, 20, 29, 57, or 85 days after *T. spiralis* infection. An initial suppression in the 14-day test group was observed; however, mice tested at later time intervals exhibited potentiation of the 24-h footpad reaction to old tuberculin over that elicited by appropriate controls. This suggested that *T. spiralis* induces a potentiation of the cellular immune response to BCG. Adoptive transfer studies support the cell-mediated nature of the observed footpad reaction and indicated that the initial suppression was not due to physiological factors preventing the expression of the footpad swelling reaction.

Infections with metazoan parasites have been reported to potentiate or depress humoral antibody responses to heterologous antigens (1, 3, 5, 7, 12). Mice infected with *Trichinella spiralis* exhibited immunosuppression of primary and secondary immunoglobulin (Ig) G responses to Japanese B encephalitis virus (3; A. S. Lubiniecki and R. H. Cypess, manuscript in preparation) and sheep erythrocytes (5). This was of limited duration and did not affect immunological memory or serum IgM levels (manuscripts in preparation). Similarly infected mice showed resistance to both intravenous and intraperitoneal infection with *Listeria monocytogenes* (4; manuscript in preparation). Since resistance to *L. monocytogenes* has been reported to be mediated by cellular immunity (10) and infection with *T. spiralis* has been shown to induce cell-mediated hypersensitivity (2), the effect of infection with *T. spiralis* on the development of cell-mediated immunity to BCG cells was investigated.

MATERIALS AND METHODS

Female ICR mice (CD-1 strain, Charles River Farms, Wilmington, Mass.), 8 to 10 weeks of age, were used throughout the study. The strain of *T. spiralis* and the methods of isolating larvae and of oral infection of mice with 200 larvae were those described by Larsh and Kent (8). The strain of BCG employed in this investigation was from the American Type Culture Collection (ATCC 1924). Bacteria for immunization were prepared from 7-day-old cultures grown in Dubos broth base (Difco Laboratories, Detoit, Mich.) supplemented with 1% Dubos medium albumin (Difco).

The immunization regimens utilized for the primary cellular immune studies are presented in Table 1. Groups of mice receiving an initial inoculum of BCG were injected with 4×10^6 viable microorganisms intravenously via the caudal vein. Selected groups were infected with 200 *T. spiralis* 14 days later. Animals were subsequently tested for delayed hypersensitive responses at 14, 20, 29, 57, or 85 days after administration of the nematode. Footpad swelling was used as a measure of delayed hypersensitivity (6). The level of immunity was assessed after challenge with 0.05 ml (1.25 mg) of old tuberculin (OT; Jensen-Salsbery Laboratories, Kansas City, Kan.) in one hind footpad, whereas the contralateral hind pad was injected with an equal volume of sterile physiological saline. The thickness of the feet was measured to the nearest 0.01 mm immediately before challenge and at specific intervals thereafter (4 and 24 h) by means of a caliper gauge (Oditest, model Odi OOT, H. C. Kroplin, Schlustern, Hessen, Germany). The absolute and percentage differences between the experimental and control footpads were calculated for individual mice, and a reaction was considered positive only when swelling in the challenged foot was at least 20% greater than that measured for the saline control.

In experiments to detect the adoptive transfer of cellular immunity, two groups of mice were immunized with 4×10^6 viable BCG as above and subsequently infected with 200 *T. spiralis* 14 days later. All

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TABLE 1. *Immunization regimen for groups of BCG- and T. spiralis-infected mice*

Group no.	Regimen				
	Inoculum	Time interval (days)	Infection	Time interval (days)	Footpad test
A	BCG	14	Sham	14	+
B	BCG	14	*T. spiralis*	14	+
C	Sham	14	*T. spiralis*	14	+
D	BCG	14	*T. spiralis*	20	+
E	BCG	14	Sham	20	+
F	BCG	14	*T. spiralis*	29	+
G	BCG	14	Sham	29	+
H	BCG	14	*T. spiralis*	57	+
I	BCG	14	Sham	57	+
J	BCG	14	*T. spiralis*	85	+
K	BCG	14	Sham	85	+

three groups were footpad-tested with OT at 14 days after the nematode administration, and their 4- and 24-h reactions were recorded.

The spleens of the donor mice were removed, and a single cell suspension was prepared by gently mincing tissue through a 60-gauge wire mesh in Dulbecco's modification of Eagle medium. Each recipient received, by intraperitoneal injection, 1.5×10^9 cells of which at least 95% were mononuclear cells. Two sets of recipient mice were employed for each group of donor animals. One group of recipients consisted of normal mice and the second was composed of animals infected with 200 *T. spiralis* 14 days prior to the transfer. All recipient mice were footpad-tested 3 days post-transfer of the donor spleen cells with 1.25 mg of OT.

RESULTS

The footpad reactions observed at 24 h after challenge with OT are shown in Table 2. Mice in groups A, E, G, and I (as defined in Table 1) inoculated intravenously with BCG and footpad-tested with OT 28, 34, 43, and 71 days later demonstrated a positive delayed-type hypersensitive response. Mice in group K, which were tested 99 days after sensitization, failed to develop a significant footpad reaction.

Mice sensitized with BCG followed by oral infection with *T. spiralis* exhibited a different pattern of footpad responses. At day 28 after BCG administration, all mice in groups B and C failed to respond to OT challenge in contrast to the BCG-positive controls (group A). After this initial suppression in footpad response at 28 days, *T. spiralis*-infected animals tested at 34 and 43 days after BCG challenge (groups D and F) developed a greater footpad response than did BCG controls in groups E and G ($P \leq 0.01$).

The footpad response in BCG- and *T. spiralis*-infected animals (group J) was still evident at day 99, when BCG controls (group K) no longer exhibited 24-h reactions to OT. In all groups, measurements of footpad thickness at 4 h after OT challenge failed to show significant increases compared to saline-injected control footpads.

Mice receiving spleen cells from BCG-immunized donors exhibited positive 24-h footpad reactions (Table 3). In contrast, mice receiving spleen cells from *T. spiralis*-infected mice with or without prior BCG administration failed to

TABLE 2. *Delayed hypersensitive footpad swelling reactions 24 h after challenge with OT*

Group no.	No. of animals positive/ group[a]	Avg (%)	*P* value[b]
A	42/42	28.2 (22.2-42.4)[c]	
B	0/41	7.3 (1.5-15.5)	$\ll 0.01$
C	0/29	1.6 (0.5-4.2)	
D	9/9	34.0 (2.80-46.3)	0.01
E	9/9	26.7 (22.6-31.7)	
F	9/9	31.7 (29.2-36.0)	<0.01
G	5/5	24.3 (20.3-26.7)	
H	9/9	28.0 (20.0-40.4)	>0.10
I	5/5	28.0 (24.6-33.5)	
J	8/8	27.2 (23.0-32.3)	<0.025
K	5/5	4.3 (4.0-4.6)	

[a] Reaction considered positive if challenged footpad exhibited 20% greater swelling than control pad.

[b] Probability that the average percent difference of the *T. spiralis* group is identical to that of the corresponding group without parasitic infection; *P* values were similar by Student's *t* test and Wilcoxen rank test for two samples.

[c] Numbers in parentheses indicate range.

TABLE 3. *Effect of T. spiralis infection of adoptive transfer of cellular immunity to BCG with 1.5×10^9 spleen cells*

Donor	Recipient	Group tested	No. positive/ no. tested	Avg (%)
BCG		D[a]	23/23	30.0 (25.4-33.8)[b]
	Normal	R	5/5	26.9 (25.6-28.6)
	T. spiralis	R	5/5	26.0 (22.1-28.6)
BCG-TS		D	0/22	6.4 (1.5-15.5)
	Normal	R	0/5	6.1 (4.2-9.1)
	T. spiralis	R	0/5	3.5 (1.9-6.0)
TS		D	0/20	2.4 (0.5-4.2)
	Normal	R	0/5	3.1 (1.9-4.2)
	T. spiralis	R	0/5	3.1 (1.9-3.9)

[a] D, Donor; R, recipient.

[b] Numbers in parentheses indicate range.

develop a footpad response. Previous *T. spiralis* infection of recipients did not appear to affect the ability to adoptively transfer cell-mediated immunity to BCG.

DISCUSSION

Results indicate that infection with *T. spiralis* produces an initial suppression followed by a prolonged potentiation of the cell-mediated immune response to BCG. The immunosuppressive phase was detected 14 days after *T. spiralis* infection but was no longer evident by day 20. This suppression was of shorter duration than the humoral immunodepression (manuscript in preparation). Adoptive transfer experiments were initiated to determine whether the diminution in footpad swelling activity at 14 days was related to a suppression of cell-mediated immune response to BCG or rather was a result of physiological factors preventing the expression of the swelling reaction. These experiments suggest that failure to develop a positive footpad reaction to OT at 14 days was due to a defect in the adoptively transferred splenic cells rather than in the physiological state of *T. spiralis*-infected recipients. The nature of this defect is currently under investigation.

On days 20, 29, and 85 after *T. spiralis* infection, a potentiation of footpad response of OT was observed in parasitized mice as compared to BCG controls. This potentiation was most evident at 85 days, when, in contrast to the positive reaction observed in parasitized mice, those mice receiving only BCG infection failed to respond to challenge with OT. On day 57, the parasitized and control mice appeared to be equally responsive to OT challenge. However, analysis of variance methods demonstrated that the OT responses of parasitized mice on days 20, 29, 57, and 85 were not significantly different; similar analysis of the responses of control mice on days 14, 20, 29, and 57 also failed to demonstrate significant differences. Statistical comparison of this data for parasitized and control mice, or the data for the two groups on days 20, 29, and 57, reproducibly showed that *T. spiralis* infection potentiated the OT response compared to that of control mice ($P < 0.05$ by the F test in both cases). Thus, the apparent lack of difference between the footpad responses of parasitized mice and control mice on day 57 may be due to random variations in these groups, and possibly should not be considered as a failure to detect potentiation. It is, of course, also possible that potentiation did not exist at 57 days, for which we have no plausible explanation. The absence of a footpad response at 4 h in any test group implied a negligible role for an Arthus type of humoral reaction in this system.

Under certain conditions, infection with *Nippostrongylus brasiliensis*, a strongylid parasite of rats, has been shown to potentiate the reagin response to previously administered immunogens (13). This potentiation was shown to be of short duration and did not involve Ig classes other than IgE (1). In contrast, our data indicate that infection with *T. spiralis* resulted in a marked alteration in the humoral as well as cell-mediated responses to heterologous antigens. This alteration was expressed as a depression of both primary and secondary IgG responses to Japanese B encephalitis virus but as a potentiation in cell-mediated response to BCG.

There are several possible explanations for the observed potentiation in cell-mediated response to BCG in *T. spiralis*-infected mice, including increased bacterial replication leading to increased antigen stimulation, a nonspecific activation of the reticulendothelial system (3), and enhancement of T cell function (11).

It is apparent from our findings that infection with *T. spiralis* resulted in a marked alteration in the humoral and cellular responses to heterologous antigens. The clinical significance of these parasite-induced alterations in the etiology of a variety of immune-mediated diseases and host responses to infectious agents and neoplasia (9, 14) remains to be resolved.

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