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Modulation of Lymphoid Cell Blastogenic Responsiveness to Mitogens by *Nippostrongylus brasiliensis* Infection (41230)

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Abstract. Spleen and mesenteric lymph node cell blastogenic responses to the mitogens concanavalin A and lipopolysaccharide and to parasite antigens were examined *in vitro* following removal from mice undergoing primary or secondary infection with *Nippostrongylus brasiliensis*. During primary infection spleen cells showed a marked increase in proliferative responsiveness to both mitogens, followed by a marked depression thereafter. During secondary infection the response of spleen cells to both mitogens remained depressed. In contrast, cells from the mesenteric lymph nodes of infected mice exhibited enhanced responsiveness to Con A and LPS, followed by depression of the response, followed by another cycle of enhancement upon reinfection. Sensitivity of both spleen and especially mesenteric lymph node cells to Nb antigens was greatest at approximately the time of worm expulsion: Day 13 after primary and Day 8 after secondary infection.

Altered immune responsiveness of lymphoid cells obtained from parasitized animals has been noted in several model systems (1). For example, mice infected with the nematode *Ascaris suum* exhibit depressed humoral and cellular immune reactivity to a wide variety of antigens unrelated to the parasite (2). Changes in responses to antigens and mitogens have also been observed over the course of infection of mice with *Trichinella spiralis* (3, 4). Studies in this laboratory have shown that inbred mice infected with the gastrointestinal nematode *Nippostrongylus brasiliensis* (Nb) exhibit markedly altered immune responses to T-lymphocyte-dependent antigens such as sheep erythrocytes (5). Following infection with Nb, mice show briefly enhanced, then persistently depressed, IgM antibody responses to sheep erythrocytes. Cellular immune responses to sheep erythrocytes are also depressed following Nb infection. In contrast to the depressed responses to heterologous antigen, specific responsiveness to parasite antigens, as manifested by expulsion of the host's intestinal worm burden, remains intact.

The mechanisms responsible for the altered immune responsiveness exhibited by parasitized hosts are not understood. The depressed immune responses observed may be related either directly to immunosuppressive substances released by the parasite or indirectly through parasite-triggered host-mediated immunomodulatory mechanisms. In the present study, lymphoid cells removed from mice at various times after Nb infection were found to exhibit markedly altered proliferative capacities when challenged with concanavalin A (Con A) or bacterial lipopolysaccharide (LPS) *in vitro*. Enhancement or depression of the proliferative response to these agents was dependent upon the stage of the infectious process and did not appear to be affected by parasite products.

Materials and Methods. *Animals.* Female BDF_1 (C57BL/6 × DBA/2 F_1 hybrid) mice were used for these studies. They were obtained from Laboratory Supply Company, Indianapolis, Indiana, and were approximately 7 to 8 weeks old at the time of infection. The mice were housed in groups of 10 and fed Purina Mouse Pellets and water *ad libitum*.

Parasite and parasite antigen. A mouse-adapted strain of Nb was obtained from Dr. N. D. Reed, Montana State University, and maintained by alternate pas-

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sage through outbred Swiss mice and feces–charcoal cultures. Parasite antigen (Nb-Met Ag) was prepared by incubation of washed adult worms, obtained from the small intestines of mice 6 days after infection, in RPMI 1640 medium containing penicillin (100 units/ml) and streptomycin (100 μg/ml) for 24 hr at 37° in a 5% CO_2 in air atmosphere. At the end of this period greater than 95% of the worms were actively motile. Antigen-containing supernatants from cultures containing approximately 1000 worms/ml of medium were collected by centrifugation and stored at −20°. Gram stains of supernatants and of pellets containing worms revealed no bacterial contamination of cultures.

Infection. Mice were injected subcutaneously in the dorsal neck region with 10^3 extensively washed (RPMI 1640 medium containing penicillin and streptomycin) filariform larvae suspended in 0.5 ml saline. Secondary infections with the same number of larvae were initiated 14 days after primary infection. Worm burden determinations were made by direct count of worms harvested from the small intestines on various days after larval injection.

Blast cell transformation assay. Single-cell suspensions of spleen and mesenteric lymph nodes (MLN) of three to six normal control and Nb-infected mice were prepared by teasing in RPMI 1640 medium supplemented with 10% fetal calf serum and containing penicillin (100 units/ml) and streptomycin (100 μg/ml). Aliquots of cell suspensions containing 2×10^5 nucleated viable cells in 0.2 ml medium were added in triplicate to flat-bottom microtiter wells in the presence of either 50 μg/ml *Escherichia coli* lipopolysaccharide (Sigma, St. Louis, Mo.), 4 μg/ml concanavalin A (Miles Laboratories, Elkhart, Ind.) or 60 μl/ml Nb-Met Ag. Preliminary studies had shown that these concentrations of mitogen gave optimal stimulation. Following 48 hr of incubation at 37° in an atmosphere of 5% CO_2 and air, the cell suspensions were pulsed with 0.5 μCi/ml methyl [^{3}H]thymidine (New England Nuclear Co., Boston, Mass.) and then harvested onto glass fiber filters with a model M-12 multiple-well cell harvester (Brandel Biomedical Research Laboratories, Rockville, Md.). Incorporation of label was measured by conventional liquid scintillation counting. Results were expressed as a stimulation index (SI) derived from the ratio of mean counts per minute of stimulated cultures relative to the mean counts per minute of unstimulated cultures. To facilitate comparison among the experiments a stimulation ratio (SR) was calculated as the ratio of SI of cell suspensions from Nb-infected mice to the SI of cells from uninfected control mice. SR values >1.0 indicated enhanced responses whereas SR <1.0 indicated depressed responses of cells from Nb-infected mice. Standard errors in replicate cultures were consistently less than 10%.

Statistical analysis. Differences among groups of experimental data were compared by the unpaired Student's *t* test. When the *P* value was less than 0.05, differences were considered significant.

Results. The number of worms present in the small intestines of mice on various days after injection of 10^3 Nb larvae increased rapidly after their first appearance on Day 4 (Fig. 1). Maximum worm numbers (approximately 25% of the injected larvae) were observed on Days 7 to 9 and worm expulsion was complete by Day 13 after primary infection. Reinfection with 10^3 larvae resulted in less than 2% of the injected

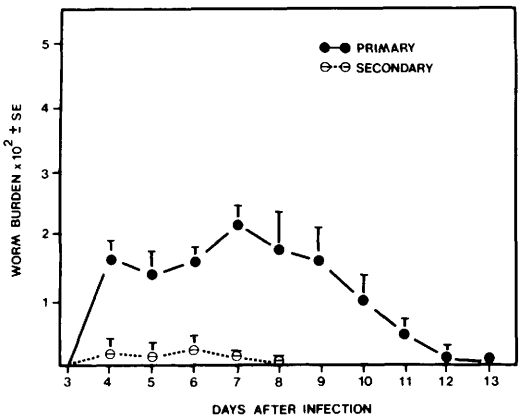

FIG. 1. Intestinal worm burdens in BDF$_1$ mice following infection with Nb. Each point indicates number of larvae ± SE in intestines of 6–10 animals at indicated time after primary (●——●) or secondary (○- - - - -○) infection with Nb.

larvae successfully colonizing the small intestine and worm expulsion was complete by Day 8 (Fig. 1).

The proliferative responses of spleen and MLN cells to stimulation with LPS or Con A varied as a function of time following either primary or secondary infection (Tables I and II). Spleen cells obtained early during primary infection (Day 4) exhibited a significantly enhanced responsiveness to Con A (SR = 2.94) (Table I). However, responses subsequently became depressed. The spleen cell responsiveness to LPS also showed significant depression (SR = 0.42) but at a somewhat later time after infection (Day 13). Following reinfection 14 days after primary infection, the response of spleen cells to Con A remained markedly depressed. The response of these cells to LPS was less affected.

In contrast, the MLN cell suspensions exhibited significantly enhanced responses to both Con A and LPS 4 and 7 days after primary infection (Table II). The responses to both mitogens subsequently became depressed. However, unlike the persistently depressed responsiveness of spleen cells, MLN cells harvested from mice following reinfection again showed enhanced responses to both Con A (SR = 2.51) and LPS (SR = 3.20).

Despite the depressed responses of spleen and MLN cells to mitogens during primary Nb infection, there was no significant depression of the proliferative response to Nb-Met Ag. As can be seen in Table III, soluble metabolic antigen present in the supernatants obtained from adult worm cultures, when incubated with spleen or MLN cells from infected animals, produced a marked enhancement of the proliferative response as a function of time after infection. Cells obtained from mice at approximately the time of expulsion of worms from a primary infection (Day 13), showed a strong response to Nb-Met Ag and an even greater response when obtained from mice after secondary infection. In particular, MLN cells from secondarily infected mice responded with an SR of 18 to 44 on Day 8, approximating the time of worm ex-

TABLE I. EFFECT OF Nb INFECTION ON PROLIFERATIVE RESPONSES OF SPLEEN CELLS TO MITOGENS

Day after infection[a]	Stimulation ratio[b] Con A	LPS
Primary infection		
4	2.94	1.32[c]
7	0.61[c]	1.14[c]
11	0.89	1.21[c]
13	0.43	0.42
20	0.61	0.81
34	0.24	0.28[c]
Secondary infection		
7	0.36	0.64[c]
11	0.23	0.30
18	0.90	0.86[c]
25	0.77	1.13[c]

[a] Spleens from groups of three to six BDF_1 mice harvested on indicated day after infection with 10^3 larvae. Secondary infections with 10^3 larvae initiated 14 days after primary infection.

[b] Cultures incubated with 4 μg/ml Con A or 50 μg/ml LPS for 48 hr, then pulsed for 24 hr with [^{3}H]-thymidine. Stimulation ratios (SR) calculated as SI cells from infected mice/SI cells from uninfected mice.

[c] Not significant ($P > 0.05$); derived from comparison of mean cpm of replicate cultures of cells from Nb-infected and uninfected mice.

TABLE II. EFFECT OF Nb INFECTION ON PROLIFERATIVE RESPONSES OF MESENTERIC LYMPH NODE CELLS TO MITOGENS

Day after infection[a]	Stimulation ratio[b] Con A	LPS
Primary infection		
4	13.70	11.50
7	4.38	1.26
11	0.23	0.94
13	0.20	1.04[c]
20	0.42	0.57
Secondary infection		
7	2.51	3.20
11	6.49	0.48
18	0.72	1.06
25	1.89	2.67

[a] Mesenteric lymph nodes from groups of three to six BDF_1 mice harvested on indicated day after infection with 10^3 larvae. Secondary infections with 10^3 larvae initiated 14 days after primary infection.

[b] Cultures incubated with 4 μg/ml Con A or 50 μg/ml LPS for 48 hr and then pulsed for 24 hr with [^{3}H]-thymidine. Stimulation ratios (SR) calculated as SI cells from infected mice/SI cells from uninfected mice.

[c] Not significant ($P > 0.05$); derived from comparison of mean cpm of replicate cultures of cells from Nb-infected and uninfected mice.

TABLE III. PROLIFERATIVE RESPONSIVENESS OF SPLEEN AND MESENTERIC LYMPH NODE CELLS TO Nb METABOLIC ANTIGENS FOLLOWING PRIMARY AND SECONDARY INFECTIONS

Day after infection[a]	Stimulation ratio[b]	
	Spleen cells	MLN cells
Primary infection		
4	1.10[c]	5.80
7	0.73[c]	3.32
11	3.28	20.60
13	8.06	19.20
20	3.80	3.40
34	0.79	—
Secondary infection		
7	3.56	18.60
11	1.31	43.90
18	3.91	3.87
25	3.17	4.56

[a] Spleen and mesenteric lymph node cells from groups of three to six BDF_1 mice harvested on indicated day after infection with 10^3 Nb larvae. Secondary infections with 10^3 larvae initiated 14 days after the primary infection.

[b] Cultures incubated with 60 μl/ml Nb-Met Ag for 48 hr and then pulsed for 24 hr with [^{3}H]thymidine. Stimulation ratios (SR) were calculated as SI cells from infected mice/SI cells from uninfected mice.

[c] Not significant ($P > 0.05$); derived from comparison of mean cpm of replicate cultures of cells from Nb-infected and uninfected mice.

pulsion. Spleen and MLN cells from uninfected mice did not respond to Nb-Met Ag when compared to medium alone. (Data not shown).

Discussion. Experiments in this laboratory have revealed marked alterations of immune responses to a T-lymphocyte-dependent antigen (sheep erythrocytes) in mice infected either primarily or secondarily with Nb (6). In the present study, the blastogenic responsiveness of spleen and mesenteric lymph node cells from mice infected once or twice with Nb were also greatly altered. Early in primary infection splenocytes exhibited enhanced responsiveness to Con A, considered a T-cell mitogen, although responses to LPS, considered a B-cell mitogen, were unchanged. However, close to the time of worm expulsion a significant depression of response to both mitogens occurred. The depressed response of T cells to Con A persisted following a secondary injection of Nb larvae. Thus it appears that splenic T lymphocytes are more subject to parasite-induced regulation than are splenic B lymphocytes.

Mesenteric lymph node cells also showed a significantly enhanced uptake of tritiated thymidine followed by a depressed response to both Con A and LPS during primary infection. During secondary infection, in contrast to the depressed responses of spleen cells, the lymph node cells again exhibited enhanced responsiveness to both mitogens. This difference may be due to the stronger restimulation by parasite products of cells residing in the draining mesenteric lymph nodes.

Results similar to these have been reported in rats infected with Nb (7). In rats undergoing a primary infection, both spleen and mesenteric lymph node cells exhibited early enhanced mitogenic responsiveness to phytohemagglutinin (PHA), a T-cell mitogen, followed by depressed responsiveness at approximately the time of worm expulsion (Day 14). Mesenteric lymph node cells harvested from rats following second infection with Nb larvae also showed a significant increase in uptake of tritiated thymidine. As in mice undergoing a secondary infection, spleen cells from reinfected rats failed to show enhanced responsiveness to PHA.

We consider it possible that alterations of responsiveness to mitogens during Nb infection may be related to the commitment of lymphoid cells to Nb antigens. From our results, and those of others (8), blastogenic responsiveness to Nb-Met Ag clearly develops very rapidly during the course of primary and secondary infections with the greatest responses being observed following the injection of a second larval inoculum. Thus, it appears plausible that the depressed responses to mitogens, especially to Con A during worm expulsion, may be related to a form of antigenic competition, i.e., antigen-induced immunosuppression. The strong responses of spleen and especially lymph node cells to Nb-Met Ag coincidental with the time of worm expulsion, a highly T-lymphocyte-dependent process (9), support this concept.

The enhanced responsiveness to mitogens occurring shortly after infection with Nb may indicate a polyclonal activation of

lymphoid cells. Infection of mice by another parasite, *Schistosoma mansoni,* also permits demonstration of the enhanced-depressed response cycle of lymphoid cells to mitogens (10). This parasite, in contrast to Nb, lives in intimate contact with its host's lymphoid system and thus makes difficult separation and distinction of direct parasite-mediated effects from indirect parasite-induced immunoregulatory activities. In the Nb system, parasite products are unlikely to be directly responsible for depressed mitogenic responsiveness since, in contrast to infections with parasites such as *S. mansoni,* worm expulsion results in a cessation of exposure to parasite antigens. Also, addition of Nb-Met Ag to cultures of spleen and lymph node cells of normal mice failed to enhance or suppress the response to mitogens. More likely, lymphoid cells, stimulated *in vivo* by worm products to undergo polyclonal activation, may release lymphokines or perhaps monokines which modulate immune responses to other antigens or mitogens. If this concept is correct then the depressed mitogenic responses observed in Nb-infected mice and rats possibly may be related to production of lymphoid cell suppressive factors which dampen the immune response nonspecifically. A wide variety of soluble factors produced by antigen- or mitogen-stimulated lymphoid cells has been found to be immunosuppressive (11). Furthermore, preliminary results obtained in this laboratory and by other workers (7) suggest that sera of infected mice or rats, when added to cultures of normal cells, suppress proliferative responses to mitogens. Thus, it appears that Nb may prove to be a useful tool in the study of parasite-induced immunomodulation.

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