DEPRESSED SPECIFIC AND NONSPECIFIC IMMUNE RESPONSES IN SECONDARY *BRUGIA PAHANGI* INFECTION IN JIRDS

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Abstract. The immune responsiveness to specific antigens or mitogens was examined in jirds after primary and secondary infections with *Brugia pahangi.* When spleen cells were obtained from secondarily infected jirds, their proliferative responses to mitogens such as Con A or LPS, or to specific antigens prepared from infective larvae or adult worms were significantly lower than those of spleen cells obtained from primarily infected jirds. The proliferative responses of the peripheral blood mononuclear cells obtained from animals undergoing primary and secondary infections also showed a similar tendency. The depressed proliferative responses of the secondary infected spleen cells to Con A or LPS was partially restored by removing adherent/phagocytic cells from the original cell populations. After deletion of the adherent cells, however, antigen-specific proliferative responses were not altered and remained at low level. These results suggest that at least two different mechanisms of depression, namely adherent cell-mediated antigen-nonspecific suppression and unresponsiveness of lymphocytes to filarial antigens, are induced in jirds in the secondary infection.

INTRODUCTION

Filariasis is a chronic infection in which the host and parasite establish a favorable relationship for many years unergoing repeated infections. In order to evade attack by the host defense mechanisms, filarial worms may affect directly or indirectly the immune system of the host to cause immune suppression (Horii *et al,* 1989, Lammie and Katz, 1983a, b, 1984a, b; Ottesen *et al,* 1977; Piessens *et al,* 1982; Portaro *et al,* 1976; Weller, 1978). Such an altered immune responsiveness of the host is thought to be important in the development of the host-parasite relationship (Barriga, 1981). Furthermore nonspecific suppression of the host's defence mechanism seems to be closely related to the development of other infectious diseases. For example, high incidence of adult T cell leukemia (ATL) antibody-positive cases were found in chronic filariasis cases in Japan (Tajima *et al,* 1983).

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Thus, elucidation of suppressor components operating during filarial infection is of great importance not only in understanding the pathobiology of filariasis but also in understanding how the host's defence mechanism to foreign antigen is altered by filarial infection. A particularly important point is to clarify the immunological events at multiple infections, because clinical manifestation of filariasis is usually caused by repeated infections. In the present study, therefore, we examined the difference in the proliferative responses of lymphocytes to specific antigens and mitogens in jirds undergoing a primary or secondary infection with *B. pahangi.*

MATERIALS AND METHODS

Animals

Outbred male Mongolian jirds *(Meriones unguiculatus)* were maintained under conventional conditions. At the beginning of each experiment they were 10 - 15 weeks of age.

Infection with parasites

Infective larvae (L3) of *B. pahangi* were ob-

tained from *Aedes aegypti* fed on infected jirds 14 days earlier. For a primary infection, jirds were infected subcutaneously in the groin by 100 L3 suspended in 0.5 ml of Hanks balanced salt solution (HBSS). For the challenge infection, jirds were infected with 100 L3 in the same manner 20 weeks after a primary infection.

Parasite antigens

Adult worms of *B. pahangi* obtained by the autopsy of infected animals were chopped finely with scissors. Infective larvae (L3) were obtained from *A. aegypti* as described above. Extraction of the parasite antigens was performed by the method described previously (Owhashi and Ishii, 1982). In short, both adults and larvae were separately suspended in phosphate buffered saline and homogenized in a glass and Teflon homogenizer placed in an ice-chilled water bath. Extraction was carried out by continuous stirring of the homogenate at 4°C overnight, and then the homogenate was centrifuged at 100,000*g* for 1 hour. The supernatant was filter-sterilized and used as the larval (L3) and adult worm (Aw) antigen. Protein concentration was determined by the method of Lowry *et al* (1951).

Cell separation

Peripheral blood samples (2 ml from each animal) were collected with 10 U/ml heparin. Lymphocytes were obtained by centrifugation (600*g* for 15 minutes at 25°C) of the blood over a Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) layer. The interface lymphocytes were removed and washed 3 times with HBSS. Spleen cell suspensions were prepared by mincing the spleen in HBSS and filtering through 4 layers of sterile surgical gauze. Splenic nonadherent cells were prepared by the method described previously (Owhashi and Nawa, 1987) with slight modification in the procedure. In short, a spleen cell suspension (2×10^6/ml) prepared in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco, Gland Island, NY, USA) was incubated in a plastic Petri dish (8 - 757 - 12, Fisher Scientific Co) at 37°C for 60 minutes in a 7% CO_2 - 93% air atmoshere. The nonadherent cells were removed and the procedure was repeated. The final suspension of nonadherent cells was washed with HBSS and resuspended in RPMI 1640 containing 3% heat-inactivated FBS. The adherent cells were recovered by gentle scraping of the dishes with a rubber policeman.

Lymphocyte proliferation assay

Spleen cells or peripheral blood lymphocytes were suspended in RPMI 1640 medium (Gibco) supplemented with 4 mM L-glutamine, 100U/ml of penicillin, 100 μg/ml of streptomycin, 2×10^{-5} M 2-mercaptoethanol and 3% heat-inactivated FBS at a concentration of 2×10^6 cells/ml, and quadruplicate 0.1 ml aliquots were placed in a 96 well flat-bottomed microtiter plate with L3 (20 μg/ml), Aw (20 μg/ml), Con A (1 μg/ml except for the dose response experiment, pharmacia) or LPS (10 μg/ml, Gibco) for 72 hours at 37°C in 7% CO_2 - 93% air atmosphere. Each well was pulsed with 18.5k Bq (^{3}H)-TdR for the last 12 hours. The cell were harvested on glass filters with a cell harvester (Dynatech) and counted in a liquid scintillation counter (LSC3500, Aloka, Japan). The experiments were repeated at least 3 times.

RESULTS

The proliferative response of spleen cells or peripheral blood mononuclear cells were compared in jirds between undergoing a primary or secondary infection with *B. pahangi*. As shown in Fig 1, the proliferative response of spleen cells obtained from jirds undergoing secondary infection was remarkably lower at all concentrations of Con A when compared with that obtained from jirds undergoing a primary infection and from uninfected controls. The proliferative response of spleen cells or peripheral blood mononuclear cells to *B. pahangi* L3 or Aw antigen was also depressed in the secondary infection (Table 1). The degree of unresponsiveness observed in the secondary infection was comparable between the responses to specific antigens and to mitogens.

To see whether the unresponsiveness observed in the secondary infection is due to the presence of suppressor cells, spleen cells obtained from jirds undergoing a secondary infection was fractionated into plastic-adherent and nonadherent cells and the proliferative response of nonadherent cell population was compared to that of unfractionated original population (Fig 2). After removal of

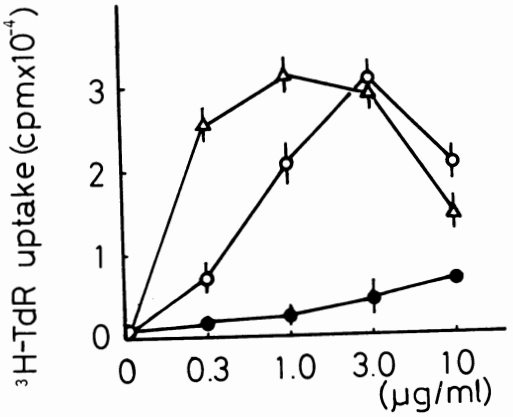

Fig 1—Proliferative response of spleen cells from primary or secondary infected jirds with *B. pahangi* or uninfected jirds to Con A. Spleen cells from primary (—○—) or scondary (—●—) infected jirds with *B. pahangi* or uninfected jirds (—△—) were stimulated with various concentration of Con A for 72 hours at 37°C. ^{3}H-TdR was pulsed for the last 12 hours. The results were expressed as the mean ± SEM of net cpm.

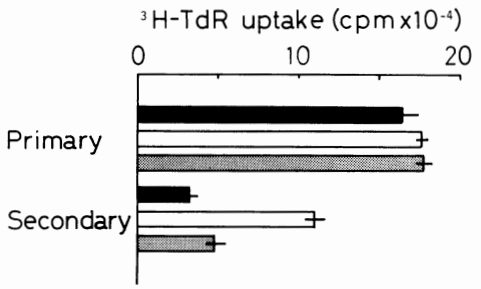

Fig 2—Comparison of the effect of adherent cells on the proliferative response between primary or secondary infected jirds. 2 × 10^6/ml of crude spleen cells (closed column), 2 × 10^6/ml of splenic nonadherent cells (open column), or 2 × 10^6/ml of splenic nonadherent cells + 4 × 10^5/ml of splenic adherent cells (dotted column) were cultured with Con A (1 mg/ml) for 72 hours at 37°C. ^{3}H-TdR was pulsed for the last 12 hours. The results were expressed as the mean ± SEM of net cpm.

Table 1

Proliferative response of spleen cells or peripheral blood lymphocytes to specific antigens in *B. pahangi*-infected jirds.

Antigen	Conc (μg/ml)	Primary[a]		Secondary[b]		
		cpm (× 10^{-2})[c]	E/C[d]	cpm (× 10^{-2})	E/C[d]	% Suppression[e]
Spleen cells						
L3	20	172 ± 2	13.2	47 ± 2	2.0	85
Ad	20	194 ± 13	14.9	47 ± 3	2.0	87
Con A	1	901 ± 24	69.3	178 ± 7	7.4	89
LPS	10	335 ± 27	25.8	69 ± 5	2.9	80
		13 ± 1		24 ± 1		
Peripheral blood lymphocytes						
L3	20	20 ± 2	3.3	7 ± 1	1	73
Ad	20	29 ± 3	4.8	5 ± 1	1	85
Con A	1	1184 ± 45	197	157 ± 6	20	90
-		6 ± 1		8 ± 2		

[a] 3 weeks after primary infection
[b] 3 weeks after secondary infection
[c] 2 × 10^6 of spleen cells or peripheral blood lymphocytes were cultured in 96 well microtiter plate for 72 hours. ^{3}H-TdR was pulsed for final 12 hours. Results were expressed as the mean ± SEM.
[d] E/C cpm of stimulated culture with antigen / cpm of unstimulated cultures.
[e] % suppresson = [1-(E/C of the secondary infected group / E/C of the primary infected group)] × 100.

adherent cells, the proliferative response of nonadherent spleen cells to Con A was significantly enhanced up to 3 times of that of the original cell population. When the adherent cells from secondary infection were added to the nonadherent cells, the proliferative response of the later was significantly suppressed. In contrast, the proliferative response of spleen cells of primary infected jirds was not altered even after the removal of adherent cells. Similarly, the splenic adherent cells from primary infected jirds had little effect when they were added to the nonadherent cells.

When similar experiments were performed using parasite antigens as the stimulant for the proliferative response (Table 2), the removal or reconstitution of the splenic adherent cells had no or. little effect on the proliferative response to L3 or Aw antigen.

DISCUSSION

The results reported here show that the proliferative responses of lymphocytes obtained from jirds undergoing secondary infection with *B. pahangi* were significantly lower than those obtained from jirds undergoing primary infection regardless of the stimulants used, namely nonspecific mitogens or specific antigens. The apparent unresponsiveness to mitogen seems not simply due to the increase in the ability of the clearance of non-self particles by phagocytic cells, because the depressed proliferative response was not recovered even when the dose of mitogen was increased to supraoptimum to the cells from uninfected or primarily infected animals (Fig 1). This fact suggests that the suppression of mitogen responsiveness in not simply due to a lack of reactive lymphocytes or to the loss of mitogen responsiveness but due to the existence of suppressive adherent cells. Rather, unresponsiveness of secondarily infected animals to mitogen is, at least in part, due to the presence of suppressive cells, because removal of adherent cells could partly restore mitogen reactivity. Presence of antigen-nonspecific immune suppressive adherent/phagocytic cells was already reported not only in filariasis (Lammie and Katz, 1983a,b) but also in schistosomiasis (Todd *et al,* 1979) or in malaria (Wyler *et al,* 1979). Our results indicate that such nonspecific suppressor macrophages would potentially be induced by repetitive infection. Thus, increase of suppressive adherent cells would be one of the main immune regulatory mechanisms during a secondary *B. pahangi*-infection in an antigen-nonspecific manner.

In the present study, the proliferative response of spleen cells from secondary infected jirds to Con A was only partially restored by removal of

Table 2

Effect of the removal of adherent cells on the proliferative response of the spleen cells from secondary infected jirds to specific antigens or mitogens.

Antigen	Conc (μg/ml)	Ad (+)[a]		Ad (−)[b]		% Increase[e]	*t*-test[f]
		cpm (× 10^{-2})[c]	E/C[d]	cpm (× 10^{-2})	E/C[d]		
L3	20	56 ± 5	2.7	42 ± 4	2.2	−19	NS
AW	20	72 ± 3	3.4	40 ± 1	2.1	−38	NS
Con A	1	110 ± 7	5.2	287 ± 16	15.1	190	$p < 0.01$
LPS	10	45 ± 9	2.1	119 ± 10	6.3	200	$p < 0.01$
		21 ± 2		19 ± 1			

[a] Spleen cells before the removal of adherent cells.
[b] Spleen cells after the removal of adherent cells.
[c] 2×10^6 of spleen cells or peripheral blood lymphocytes were cultured in 96 well microtiter plate for 72 hours. ^{3}H-TdR was pulsed for final 12 hours. Results were expressed as the mean ± SEM.
[d] E/C = cpm of stimulated culture with antigen / cpm of unstimulated cultures.
[e] Percent increase = [E/C Ad(−) /E/C Ad(+) −1] × 100.
[f] The data were compared statistically by Studen's *t* test.

the adherent cells (Fig 2). These results suggest that at least two different immune regulatory events, adherent cell-mediated and nonadherent cell-mediated mechanisms, are involved in the suppression of mitogen reactivity in the secondary infection. Related to this, we recently showed the existence of $CD8^+$ antigen-nonspecific suppressor T cells, which could suppress proliferative response to mitogen, in *B. pahangi*-infected Lewis rats (Owhashi *et al,* 1990).

In contrast to the mitogen response, antigen-specific proliferative response to L3 or Aw antigen remained low even after the removal of adherent cells. This result suggests that the proliferative response to specific antigens is under regulation of different mechanisms from those to Con A or LPS. Concerning this, Lammie and Katz (1984a,b) showed the existence of antigen specific suppressor T cells in *B. pahangi*-infected jirds. Recently King *et al,* (1992) demonstrated the decrease of the parasite-specific T and B lymphocyte precursor frequency in microfilaremic patients. This may imply the possibility that the increase of suppressive adherent cells would result in the suppression of the clonal expansion or clonal anergy of the antigen- specific T cells in a secondary infection.

Thus, as an overall conclusion, in addition to the antigen-nonspecific suppressive adherent cells demonstrated in this paper, antigen-nonspecific suppressor T cells and antigen-specific suppressor T cells may also play an important role in the immune regulatory mechanisms in the secondary infection. Mechanisms of the antigen-specific suppression, the role of the antigen nonspecific suppression and the actual effect of immune suppression on the evasion of the invading larvae from the host defense mechanisms in the secondary infection should be clarified in the future.

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