Down Regulation of Macrophage Activation in *Brugia pahangi*-Infected Jirds (*Meriones unguiculatus*)

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The macrophage is a major component of the inflammatory response induced by lymphatic tissue-dwelling filariae. Intraperitoneal (i.p.) infections with *Brugia pahangi* in Mongolian gerbils, or jirds (*Meriones unguiculatus*), induce a peritoneal inflammatory response characterized by accumulation of numerous macrophages and fewer eosinophils. This inflammatory response is associated with the release of microfilariae by female worms. The aim of this study was to investigate the activation state of the peritoneal macrophages during the course of i.p. infections with either male or female worms. Activation was determined by a toxoplasmacidal assay and assays which measured the production of tumor necrosis factor (TNF)-like activity and nitric oxide (NO) production. The development of these assays with jirds was initially conducted in parallel with the mouse system, which served as a positive control. Jird macrophages became activated to kill *Toxoplasma gondii* by in vivo immunization with *Mycobacterium bovis* BCG in a pattern similar to that of mouse macrophages. However, unlike the mouse system, supernatants from purified protein derivative- or concanavalin A-stimulated jird splenocytes plus lipopolysaccharide failed to activate jird macrophages in vitro or induce NO production. These results indicate that factors involved in jird macrophage activation may differ from those demonstrated in the mouse system and other systems. i.p. infections of 15 days in duration with either male or female worms induced macrophage activation as measured by *Toxoplasma* killing and TNF production. These responses decreased as the infection progressed to the chronic period on a time course that parallels the down regulation of experimental *B. pahangi* granulomas. There was no evidence of NO production by activated jird macrophages. These data indicate that macrophage function is down modulated during filarial infection and suggest that mechanisms involved in macrophage deactivation are related to those that induce down modulation of the systemic granulomatous inflammatory response in the jird. This response is not dependent on the microfilarial stage of the parasite and is also independent of mechanisms which induce peritoneal accumulations of macrophages.

Wuchereria bancrofti and *Brugia malayi* are lymphatic tissue-dwelling filarial nematodes that infect humans in tropical and subtropical regions of the world. The pathology caused by filarial parasites is primarily characterized by a granulomatous inflammation response to the parasite and parasite products that has been attributed to a state of specific filarial immune hyperresponsiveness (35, 46). Nevertheless, a larger group in the infected population is asymptomatic despite its members having microfilariae (MF) in their peripheral blood and a tendency to manifest a state of filaria-specific hyporesponsiveness (25, 45, 47, 49).

In *Brugia pahangi*-infected jirds, the immune response has been implicated in the development of lymphatic granulomas. Presensitization and protective immunity result in an increase of these lymphatic lesions (28, 48). Chronic microfilaremic gerbils manifest a state of hyporesponsiveness (32) accompanied by decreased lymphatic lesion numbers (27, 29) and down modulation of the granulomatous response to antigen-coated beads embolized in the lungs (PGRN) (27).

The macrophage is a major cellular component of the granuloma and is often present covering the surfaces of filariae (23, 42, 44) and other nematodes (24). The role of the macrophage in the immune response to metazoan parasites has been thoroughly investigated in murine schistosomiasis, in which it has been shown that macrophage activation is important in resistance (4, 20, 21, 37). Furthermore, several studies suggest that the macrophage is important in the immunomodulation observed during schistosoma infection (reviewed in reference 58).

The purpose of the experiments described in this paper was to determine the state of macrophage activation in *B. pahangi*-infected jirds at times when systemic granulomatous modulation occurs. The potentially different effects of female worms and MF and of male worms on macrophage function were also investigated.

MATERIALS AND METHODS

Animals. Inbred, female, 6- to 8-week-old jirds (*Meriones unguiculatus*) were obtained from Tumblebrook Farms (West Brookfield, Mass.). Inbred, female, BALB/c mice were originally obtained from Jackson Laboratories (Bar Harbor, Maine). Animals were maintained on standard rodent chow and water ad libitum. All jirds and mice used in these experiments were infected at 3 to 4 months of age.

In vivo infection with *Mycobacterium bovis* BCG. Briefly, one group of 24 jirds and one group of 24 mice were inoculated intradermally (i.d.) with 3×10^6 CFU of living *M. bovis* bacillus Calmette-Guérin (BCG). Twenty-four and forty-eight hours before euthanasia, BCG-immunized animals were inoculated intraperito-

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neally (i.p.) with 50 μg of purified protein derivative (PPD) of the tubercle bacillus (Parke-Davis, Rochester, Mich.) dissolved in 0.5 ml of phosphate-buffered saline (PBS). The BCG-PPD immunization protocol and kinetics of macrophage activation by this method have previously been described for mice (53). Control animals, 24 per group, were inoculated i.d. with 0.05 ml of PBS and i.p. with 0.5 ml of PBS. Necropsies were performed at 15, 28, and 42 days postinfection (dpi) with BCG. These time points were chosen to correspond to periods of macrophage activation in mice following BCG injection (53) and points of differential regulation of the granulomatous inflammatory response by i.p. infections of jirds with *B. pahangi* (41).

Brugia parasites. The *B. pahangi* life cycle was maintained in *Aedes aegypti* and jirds as previously described (29). Male and female adult *B. pahangi* worms were aseptically collected from the peritoneal cavities of jirds with patent i.p. infections. The worms were washed in RPMI 1640 medium supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml) prior to being transferred to 3-ml syringes. Single-sex implantations of 10 female or 10 male worms into the peritoneal cavities of jirds were done with 16-gauge needles. Control animals were inoculated i.p. with RPMI medium. Necropsies were performed at 15, 50 to 56, and 135 dpi. These time points were chosen to correspond to periods of maximal (14 dpi) and decreased (50 to 56 and 135 dpi) periods of granulomatous inflammation in response to *B. pahangi* antigen (41).

Macrophage culture. Peritoneal cells were aseptically collected from the peritoneal cavities of jirds and mice in PBS containing 10 U of heparin (Sigma) per ml. Peritoneal cells were washed once at $250 \times g$ for 10 min and transferred to RPMI 1640 (GIBCO, Grand Island, N.Y.) supplemented with antibiotics (described above), HEPES buffer (25 mM), 2-mercaptoethanol (2×10^{-5} M), L-glutamine (2 mM), and 10% heat-inactivated fetal bovine serum. Cells were prepared on LUX coverslips (Miles Scientific, Division of Miles Laboratories, Inc., Naperville, Ill.) in 24-well tissue culture plates (GIBCO) at 3×10^6 cells/well and were incubated at 37°C. After 4 to 6 h of culture, nonadherent cells were removed by washing the coverslips with PBS. Macrophage monolayers on the coverslips were used in the bioassays for *Toxoplasma* killing and for tumor necrosis factor (TNF) and nitric oxide (NO) production.

Spleen cell culture and collection of macrophage activating factors (MAF). MAF were collected from cultures of spleen cells from BCG-PPD-inoculated animals or from untreated animals.

Single-cell suspensions were obtained from spleens of BCG-PPD-inoculated and untreated animals as described previously (29). Spleen cells were cultured in 5% fetal bovine serum–RPMI 1640 medium supplemented as indicated above. To obtain BCG-PPD MAF, spleen cells were incubated at a concentration of 4×10^6 cells/ml with 40 μg of PPD per ml in 25-cm^2 plastic tissue culture flasks (Costar, Cambridge, Mass.) (40). After 48 and 72 h, culture supernatants were collected, centrifuged at $10,000 \times g$ for 10 min, and stored at −20°C until use.

To obtain concanavalin A (ConA)-induced MAF, spleen cells were incubated at a concentration of 1.5×10^6 cells/ml with 1, 2, or 3 μg of ConA (Sigma) per ml in 25-cm^2 plastic tissue culture flasks for 24, 48, 72, 96, or 120 h. Culture supernatants were collected, centrifuged at $10,000 \times g$ for 10 min, and stored at −20°C until use. Previous work in our laboratory and by other investigators (32) has demonstrated that 3 μg of ConA per ml induces spleen cell proliferation as measured by tritiated thymidine incorporation.

Reagents. Bacterial lipopolysaccharide (LPS) from *Escherichia coli* O111.B4 was used (Sigma). Recombinant murine gamma interferon (rIFN-γ) was obtained from Genentech, Inc. (South San Francisco, Calif.).

In vitro activation of macrophages. Either BCG-PPD MAF, ConA MAF (1:1, 1:2, or 1:4 MAF/medium ratio), or murine rIFN-γ (500 or 1,000 U/ml) with 10 or 50 ng of LPS per ml was added to the macrophage monolayers. Macrophages were cultured undisturbed overnight. Attempts to activate jird and mouse macrophages in vitro were repeated at least three times for each treatment.

Toxoplasma killing assay. Tachyzoites of *Toxoplasma gondii* RH were harvested from the peritoneal cavities of BALB/c mice 2 days after infection and purified by filtration through 3-μm-pore-size polycarbonate membranes (Nuclepore Corp., Pleasanton, Calif.) as described previously (54, 61). Macrophage monolayers from controls and from animals infected with BCG-PPD or *B. pahangi* were challenged with 1.5×10^6 freshly harvested *T. gondii* cells. One hour later, extracellular *T. gondii* cells were washed off and macrophage monolayers were reincubated. Microbicidal activity was assessed after 20 h of culture. Coverslips were fixed and stained with Hema 3 (Curtin Matheson Scientific, Inc., Houston, Tex.). The numbers of intracellular *Toxoplasma* cells were counted in 100 macrophages per coverslip. Triplicate samples were examined for each treatment.

TNF-like activity. Levels of TNF-like activity were determined in supernatants of macrophage cultures by a modified L929 fibroblast cell lytic assay (2, 55). An antibody that specifically neutralizes jird TNF is not available. Thus, it can only be presumed that cytolysis of L929 cells by macrophage supernatants was due to TNF. However, for the purposes of this paper this factor will be referred to as TNF. Macrophage monolayers were stimulated with 50 μg of LPS (Sigma) per ml in medium alone. Supernatants were collected after 4 h, centrifuged at $10,000 \times g$ for 10 min, and stored at −70°C until use. Duplicate samples were serially diluted threefold in 96-well, flat-bottomed tissue culture plates (Costar). L929 cells were added, and plates were incubated at 37°C. In the absence of a jird recombinant TNF standard, 100% cell lysis was accomplished with 3.0 M gua-

nidine hydrochloride. Concentrations of TNF were calculated in units defined as the reciprocal dilutions of supernatants which yield 50% lysis of L929 cells.

Measurement of nitrite production. Levels of nitrite (NO_2^-) in macrophage supernatants were determined spectrophotometrically at 540 nm following reaction with the Griess reagent (1, 10). NO_2^- is the stable end product of nonenzymatic degradation of NO. NO_2^- levels were measured in supernatants from macrophage monolayers treated in vitro with either IFN-γ or ConA MAF and LPS as second signal or with only LPS. NO_2^- production was also determined in cultured macrophages from BCG-PPD-immunized animals. NO_2^- concentration was calculated from a NaNO$_2$ standard curve, and results are expressed as micromolar concentrations.

Statistical analysis. When deemed necessary, results were analyzed statistically with a comparative analysis of variance with Tukey's Studentized range test.

RESULTS

Characterization of jird macrophage activation by *Toxoplasma* killing. *Toxoplasma* killing is a sensitive, well-defined assay used to study macrophage activation (1, 54, 55). In order to validate the use of jird macrophages in this assay, parallel comparisons of murine and jird macrophage activity were conducted by standard in vitro methods of activation. Macrophages were activated in vivo by BCG inoculation demonstrated to be effective in mice (53).

Experiments with murine macrophages treated with mouse ConA MAF or murine rIFN-γ consistently showed activation (Table 1). However, in parallel, all attempts to activate jird macrophages in vitro with supernatants from ConA-stimulated spleen cultures or with murine rIFN-γ as a first signal, followed by LPS as a second signal, failed (Table 1).

Alternatively, we chose to activate macrophages in vivo with BCG and test them ex vivo as previously demonstrated in the murine system (53). Results showed that macrophages recovered from BCG-PPD-treated mice and jirds restricted the intracellular growth of *Toxoplasma* cells compared to control macrophages at the three times tested (14, 27, and 42 dpi) ($P < 0.05$). Microbicidal activity of BCG-PPD-treated peritoneal macrophages was manifested by a decrease in the percentages of *Toxoplasma*-infected macrophages, a decrease in the numbers of *Toxoplasma* cells per infected macrophage, and a decrease in the numbers of *Toxoplasma* cells per 100 macrophages (Fig. 1). Toxoplasmacidal activity did not vary significantly in mice or jirds throughout the infection, although activated jird macrophages appeared to have a lower capacity to restrict *Toxoplasma* growth than did mouse macrophages.

TABLE 1. Effects of ConA MAF and murine rIFN-γ on the toxoplasmacidal activities of jird and mouse macrophages[a]

Macrophage	% Infected macrophages[d]	No. of toxoplasmas/infected macrophage	No. of toxoplasmas/100 macrophages[d]
Jird			
Control[b]	22.7 ± 7.13	3.18	72 ± 22.6
Murine rIFN-γ plus LPS	17.33 ± 0.94	3.35	58 ± 10.98
Jird ConA MAF plus LPS	22.67 ± 4.03	3.31	75 ± 12.35
Mouse ConA MAF plus LPS	21.5 ± 1.5	3.97	85.5 ± 15.5
Mouse			
Control[b]	15.57 ± 0.94	6.64	104 ± 15.77
Murine rIFN-γ plus LPS	3.67 ± 2.05[c]	1.27[c]	4.67 ± 2.86[c]
Jird ConA MAF plus LPS	9 ± 1[c]	4.22[c]	38 ± 5[c]
Mouse ConA MAF plus LPS	4 ± 0.82[c]	1.15[c]	4.6 ± 0.94[c]

[a] LPS was added as a second signal.
[b] Peritoneal macrophages from control uninfected animals that had no in vitro treatment.
[c] Significant difference ($P < 0.05$) from controls.
[d] Results are expressed as means ± standard deviations.

A.

B.

The production of NO by macrophages was examined further with cells recovered from BCG-sensitized jirds and mice. The greatest levels of NO$_2$ were measured in cultures of BCG-stimulated murine macrophages at 12 h of culture when stimulated in vitro with LPS (Table 3). NO production above control levels was not found in macrophages from BCG-PPD-immunized jirds.

Macrophage activation after i.p. infection of jirds with adult female or male *B. pahangi*. Previous studies have demonstrated that in vivo granulomatous inflammatory responses of jirds to *B. pahangi* antigen are stimulated by 15 days after i.p. infection with female or male worms. Conversely, infection with female worms but not with male worms induces a persistent peritoneal exudate of predominantly macrophages during this period (41). In order to characterize these changes in inflammatory events at the cellular level, the state of macrophage activation was determined by the toxoplasmacidal assay. Time points for peritoneal macrophage collection during the course of *B. pahangi* infection were chosen to coincide with the changes seen in filaria-induced inflammation.

Toxoplasmacidal activity was measured in peritoneal macrophages from jirds with i.p. infections with either adult female or male *B. pahangi*. Macrophages from uninfected jirds served as negative controls, and macrophages from BCG-PPD-immunized jirds served as positive controls. Macrophages from jirds infected with adult female or male *B. pahangi* at 15 dpi restricted the growth of *Toxoplasma* cells in a manner similar to that of macrophages from BCG-PPD-immunized jirds. Percentages of infected macrophages, numbers of *Toxoplasma* cells per infected macrophage, and total numbers of *Toxoplasma* cells per 100 macrophages at 15 dpi were significantly decreased with respect to control macrophages ($P < 0.05$) (Fig.
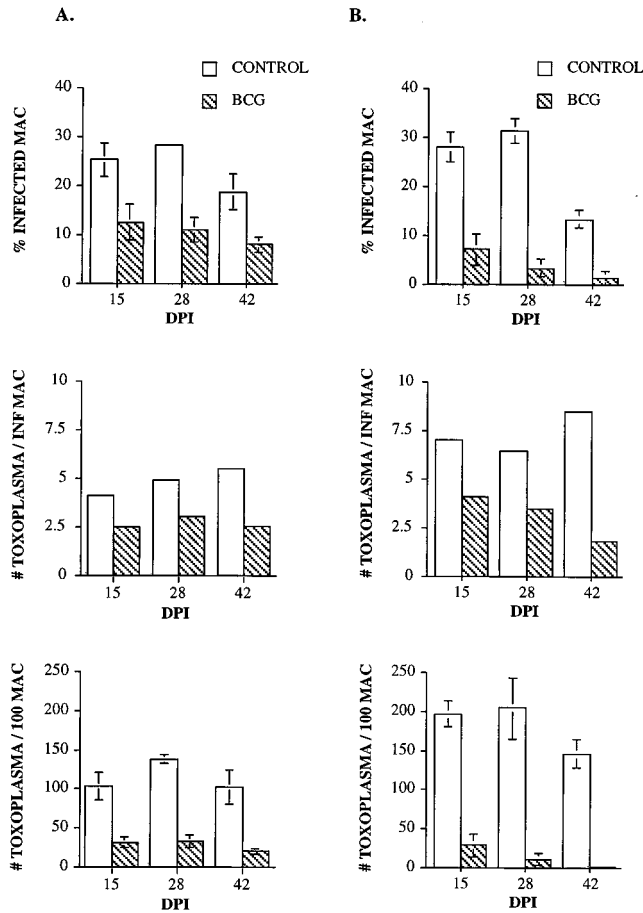
FIG. 1. Effect of BCG-PPD immunization on jird (A) and mouse (B) macrophage activation measured by the percentages of *Toxoplasma*-infected macrophages, numbers of *Toxoplasma* cells per infected macrophage, and total numbers of *Toxoplasma* cells in 100 macrophages. DPI indicates days after i.d. BCG inoculation. Bars represent standard deviations of the means.

Because of the success of in vivo activation of macrophages with BCG, attempts were made to activate macrophages in vitro with MAF obtained from these in vivo-activated macrophages. The addition of murine BCG-PPD MAF to macrophage monolayers from control mice resulted in a marked increase of microbicidal activity measured via *Toxoplasma* killing (Table 2). However, jird BCG-PPD MAF did not restrict the growth of *Toxoplasma* in macrophages from control jirds.

Nitrite production. NO is a known important effector molecule produced by activated macrophages and has been demonstrated to be important in killing of filariae in mice (50). Thus, we chose to measure the production of NO by jird macrophages stimulated both in vitro and in vivo. As for the *Toxoplasma* killing assay, results obtained from jird cell culture were compared with those from similarly treated mouse cells. NO$_2^-$ release was not detected in any of the supernatants from jird macrophage cultures treated with either ConA MAF or IFN-γ plus LPS or LPS alone or not treated. However, parallel experiments with mouse ConA MAF or IFN-γ on murine macrophages resulted in NO$_2^-$ production as previously described (9). NO$_2^-$ production increased over time in LPS-treated murine macrophages from 1.5 ± 2.1 μM at 4 h to 39.9 ± 2.3 μM at 24 h of culture. The greatest NO$_2^-$ production was observed in murine macrophages treated with a combination of IFN-γ and LPS (52.5 ± 1.3 μM) after 20 h.

TABLE 2. Effects of BCG-PPD MAF on the toxoplasmacidal activities of jird and mouse macrophages[a]

Macrophage	% Infected macrophages[b]	No. of toxoplasmas/infected macrophage	No. of toxoplasmas/100 macrophages[b]
Jird			
Set at first time			
Control	28.3 ± 0.47	4.91	139 ± 5.43
15-dpi BCG MAF plus LPS	21 ± 0.82	4.03	84.7 ± 14
Set at second time			
Control	15.7 ± 1.25	4.29	67.3 ± 3.4
28-dpi BCG MAF plus LPS	15.5 ± 0.5	4.45	69 ± 7
42-dpi BCG MAF plus LPS	12.7 ± 1.69	3.87	49 ± 8.64
Mouse			
Set at first time			
Control	31.3 ± 2.49	6.5	204 ± 39
15-dpi BCG MAF plus LPS	7 ± 5^c	1.28^c	9 ± 7^c
Set at second time			
Control	13.3 ± 1.7	8.5	146.7 ± 18
28-dpi BCG MAF plus LPS	1 ± 0^c	1^c	1 ± 0^c
IFN-γ plus LPS	1 ± 0^c	1^c	1 ± 0^c

[a] LPS was added as a second signal. BCG-PPD MAF from animals with 15, 28, and 42 days of BCG infection were used.
[b] Results are expressed as means ± standard deviations.
[c] Significantly different ($P < 0.05$) from the controls.

TABLE 3. NO production by macrophages from jirds
and mice immunized with BCG-PPD[a]

			Production by macrophage			
dpi[b]	TX[c]	ITX[d]	Mouse		Jird	
			Control[e]	BCG-PPD[f]	Control[e]	BCG-PPD[f]
15	0	4	1.29 ± 0.51	2 ± 0.8	2 ± 0.88	0.65 ± 0.7
	LPS	4	1.5 ± 0.39	2.4 ± 0.3	1.7 ± 0.5	0.8 ± 0.5
	0	12	ND[g]	13.4 ± 14	1.3 ± 0	1.12 ± 1.2
	LPS	12	5.5 ± 0.8	22.7 ± 6.6	0	0.9 ± 1.28
28	0	4	0.15 ± 0.22	1.16 ± 0.7	1.4 ± 0.6	1.4 ± 0.76
	LPS	4	ND	3.33 ± 1.4	0.8 ± 0.5	1.46 ± 0.42
	0	12	ND	0.17 ± 0.19	0	0.4 ± 0.44
	LPS	12	ND	14.6 ± 4.4	0.3 ± 0.29	0.29 ± 0.38
42	0	4	0.32 ± 0.45	4.6 ± 4.3	0.4 ± 0.5	0.54 ± 0.94
	LPS	4	0.63 ± 0.6	4.7 ± 6.7	0.17 ± 0.3	0.8 ± 0.89
	0	12	ND	14.3 ± 13.9	0	0
	LPS	12	ND	34.8 ± 20.8	0	0

[a] Results are expressed as micromolar concentrations of NO_2^- (means ± standard deviations).
[b] Number of dpi with BCG.
[c] Macrophages were cultured with or without LPS.
[d] Hours of culture with or without LPS.
[e] Uninfected animals.
[f] Animals were infected with BCG and boosted i.p. with PPD, 24 h and 48 h before euthanasia.
[g] ND, not determined.

2). In contrast, macrophages from female or male worm infections at 50 or 135 dpi showed percentages of infected macrophages, numbers of mean *Toxoplasma* cells per infected macrophage, and total numbers of *Toxoplasma* cells per 100 macrophages significantly higher than those of macrophages from BCG-PPD-immunized jirds and not significantly different from those of uninfected controls ($P > 0.05$) (Fig. 2).

TNF-like production by peritoneal macrophages from jirds infected with adult female or male *B. pahangi*. In the murine system, TNF alpha (TNF-α) has been demonstrated to play an active role in macrophage activation and serves as an inducer of many other inflammatory events. The production of this important inflammatory cytokine during the induction and regulation of macrophage activation was estimated in *B. pahangi*-infected jirds by the L929 lytic assay. Spontaneous release of TNF above controls occurred erratically in macrophages from female and male worm-inoculated jirds throughout infection. Release of TNF from LPS-stimulated macrophages was markedly elevated with respect to uninfected controls at 15 and 56 dpi in the female and male worm infections, and this response was down modulated at 135 dpi (Table 4).

DISCUSSION

Most macrophage activation systems consist of IFN-γ as a first signal, with a second signal represented either by the endogenous production of TNF-α (13, 33, 55) or by that of an exogenous factor (such as LPS) that acts independently of IFN-γ production (55) or induces TNF-α production (12). Macrophage activation in the murine system has been exhaustively studied. However, extrapolation to other species has not always been successful (9, 30, 52, 60). In the present studies, activation of jird macrophages was not accomplished by standard in vitro methods routinely employed in the murine and other systems. Parallel in vitro experiments in mice resulted in macrophage activation, indicating that these methods and re-

agents are effective in our laboratory. However, BCG-PPD immunization activated jird and murine macrophages to kill *Toxoplasma* cells when tested ex vivo.

The reasons why jird macrophages could not be activated in vitro to kill *Toxoplasma* via MAF are at this moment uncertain. If the murine model is the "gold standard" for analyzing NO-enhanced microbicidal effects in vitro, the jird is not the only species that does not conform with accepted dogma. Human macrophages treated with combinations of IFN-γ and TNF-α or LPS in vitro are unable to kill or inhibit *Mycobacterium leprae* (30), *Mycobacterium tuberculosis*, or other mycobacteria (3, 9, 52, 60), although similar treatment of human macrophages in vitro will activate them to kill other intracellular pathogens such as *Leishmania* (9, 18, 39) and *Toxoplasma* (30) cells. These differences in cell responses between and within animal species indicate that the signaling networks that lead to macrophage activation may be more complex than was first believed.

Furthermore, the fact that the NO pathway cannot be demonstrated consistently in human macrophages (review in reference 5) has also raised doubts about the concept of macrophage activation as defined in the murine system. The NO pathway has been implicated as the primary effector mechanism mediating cytotoxicity of activated macrophages (43). NO was not produced under any circumstance by jird macrophages. However, parallel experiments with murine macrophages demonstrated NO generation as previously reported by other investigators (1, 8, 14, 16, 19). In light of the recent observations on the importance of NO to *Brugia* killing (50) in murine models, it is interesting to speculate that the lack of NO production by jird macrophages is an important factor in the unique susceptibility of these rodents to filariae and other parasites.

Our results demonstrate that peritoneal macrophages from jirds infected with female or male *B. pahangi* were toxoplasmacidal at 15 dpi but not at 50 and 135 dpi. Macrophage activation in early *B. pahangi* infection coincided with the peak in PGRN to *Brugia* antigens observed in previous studies (41). The loss of toxoplasmacidal activity in peritoneal macrophages later in the course of infection corresponds to down regulation of the PGRN. At 135 dpi, no difference in macrophage microbicidal activity was demonstrated between female and male *B. pahangi* infections, indicating that the presence of MF, which are produced by the females, was not required for mac-
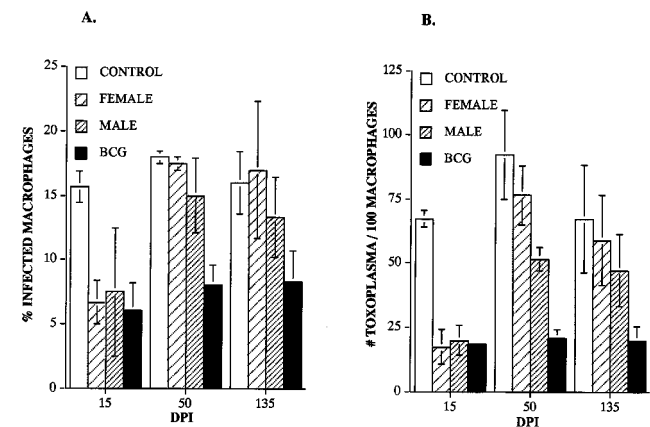

FIG. 2. Macrophage activation in jirds inoculated i.p. with female or male *B. pahangi* measured by the percentages of *Toxoplasma*-infected macrophages (A) and the mean numbers of *Toxoplasma* cells per 100 macrophages (B). Bars represent standard deviations of the means.

TABLE 4. TNF-like production by peritoneal macrophages from jirds with female or male *B. pahangi* infection[a]

Infection	15 dpi		56 dpi		135 dpi	
	No LPS	LPS	No LPS	LPS	No LPS	LPS
Female worm	12.3 ± 0.2	95 ± 73.8	0.9 ± 1.4	173 ± 83	7.7 ± 4.6	42 ± 9.5
Male worm	3.6 ± 0.8	24 ± 2.5	3.2 ± 0.6	138 ± 57	2.3 ± 2.3	5.5 ± 2.3
Control	2.4 ± 0.04	8.7 ± 0	1.2 ± 0.9	7.14 ± 4	3 ± 0.18	6 ± 3.7

[a] Macrophage monolayers were untreated (no LPS) or stimulated with 50 ng of LPS for 4 h. TNF-like activity was quantitated with the L929 cell line and expressed as units, defined as the reciprocals of the supernatant dilutions which produced 50% lysis of L929 cells (means $\pm$ standard deviations). dpi, days postinfection with *B. pahangi*. Control, uninfected control.

rophage deactivation. Similarly, previous studies (41, 42) indicate that down regulation of the PGRN is not dependent on the presence of MF. A state of filarial immune hyporesponsiveness with an absence of marked inflammation has been associated with a shift to the Th2 cell phenotype in humans (26, 38). The lack of toxoplasmacidal activity of macrophages in chronically *B. pahangi*-infected jirds may be related to the presence of Th2 cytokines that deactivate macrophages, such as interleukin 4 (IL-4) (17, 34) and IL-10 (6, 7, 56). These cytokines exert a negative effect on proinflammatory molecules, which would explain the depressed PGRN found previously. The temporal expression of cytokines in the jird during the course of *B. pahangi* infection and their potential regulatory effects on inflammation are yet to be determined.

Female worm infection resulted in a high accumulation of peritoneal macrophages, presumably due to the continuous release of MF, which act as a potent inflammatory stimulus (41). Interestingly, these macrophages were not activated to kill *Toxoplasma* and rarely formed granulomas. As has been shown in diverse macrophage-parasite interactions (reviewed in reference 51), defects in macrophage effector functions may result in suppressive effects on other immune cells. For instance, macrophages can be induced to secrete IL-10, transforming growth factor β, and prostaglandin E$_2$, which down regulate cell-mediated immunity and may drive the immune response to a Th2 phenotype. Molecules from filarial nematodes that may exert a direct effect on macrophage function have not been well studied. However, MF have been shown to release prostaglandin E$_2$ (36). This inflammatory molecule has been demonstrated to be a potent immune modulator suppressing macrophage (56, 57, 59) and lymphocyte (11) functions.

Previous studies demonstrated that macrophages from *B. pahangi*-infected jirds with chronic infections were activated to kill *Staphylococcus aureus* (22). These results may differ from the current data because the stage initiating infection was L3, which in the chronic phase resulted in the greatest macrophage accumulation with characteristic granuloma formation (23). On the other hand, the immunological requirements to kill the obligate intracellular protozoan *Toxoplasma* clearly vary from those normally required to kill the extracellular bacterium *S. aureus*. Microbicidal activity to kill *S. aureus* by macrophages from L3 *B. pahangi*-infected jirds was similar to that of thioglycolate-elicited control macrophages. However, attempts to obtain toxoplasmacidal activity in thioglycolate-elicited macrophages failed (data not shown). Killing of *Toxoplasma* organisms probably requires different immune system-mediated signals than does killing of facultative organisms such as *S. aureus*. Other investigators have demonstrated variations in the ability of macrophages to cope with organisms of more similar background (18, 31, 39).

An increase in spontaneous and LPS-induced TNF production above that of controls occurred at 15 dpi in both female and male worm infections, corresponding to macrophage activation. LPS-induced TNF production peaked at 56 dpi and decreased markedly at 135 dpi in both female and male infections. The peak in TNF production occurred at the moment macrophages were accumulating in large numbers, especially in the female infection, and may be related to the potent chemotactic function of this cytokine. The subsequent decrease could have been induced by similar factors that caused macrophage deactivation. It has been demonstrated that deactivating cytokines such as IL-10 and IL-4 inhibit production of TNF-α (6, 15). These cytokines may be more abundant or may exert a more intense down-regulatory effect as the infection progresses to the chronic time period.

We have demonstrated that the parasite-specific hyporesponsive state defined for jirds infected with *B. pahangi* may be associated with a defect in macrophage function that is manifested as an incapacity to kill *Toxoplasma* and to produce TNF-like molecules. The macrophage is recruited locally in large numbers in response to MF and may prove to be a key effector cell implicated in the immunoregulatory mechanisms that determine disease outcome in filariasis.

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Editor: J. M. Mansfield