Resolution of an Infection with *Leishmania braziliensis* Confers Complete Protection to a Subsequent Challenge with *Leishmania major* in BALB/c Mice

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Both *Leishmania major* and *L. braziliensis* induce cutaneous leishmaniasis in BALB/c mice. Whereas BALB/c mice die of infection with *L. major*, they cure an infection with *L. braziliensis*. We report here that after curing an infection with *L. braziliensis*, BALB/c mice are resistant to challenge with *L. major*. When challenged with *L. major*, *L. braziliensis* pre-treated BALB/c mice mounted a delayed-type hypersensitivity response to *L. major* and produced high amounts of interferon-*g* (IFN-*g*) but low amounts of interleukin-4. The IFN-*g* produced by the *L. braziliensis* pre-infected mice was involved in the protection seen against *L. major* challenge since treating the mice with a neutralizing anti-IFN-*g* abrogated the protection. This suggests that cross-reactive antigen epitopes exist between *L. braziliensis* and *L. major* and that pre-infection with *L. braziliensis* primes BALB/c mice to epitopes on *L. major* that can elicit a protective Th1 response to the parasite.

Key words: *Leishmania braziliensis* - *Leishmania major* - mice - cross-protection - cytokines

Organisms of the genus *Leishmania* induce a spectrum of diseases in humans and in experimental animals. Infection of mice with *L. major*, one cause of cutaneous leishmaniasis, is perhaps the best studied model for cutaneous leishmaniasis (reviewed in Bogdan et al. 1993, Liew & O'Donnell 1993, Reed & Scott 1993, Titus et al. 1994, Reiner & Locksley 1995). Most mouse strains cure an infection with *L. major*, however BALB/c mice are a notable exception since they ultimately die of infection with *L. major* when the disease becomes systemic. Considerable work in this model has revealed that mice that are resistant to infection with *L. major* develop a Th1 immune response and its associated cytokine profile [interferon-gamma (IFN-g)^{hi} Leishmania (Lehn et al. 1989, Liew et al. 1989).

In contrast to infection with *L. major*, *L. braziliensis* induces only a transient cutaneous disease, even in BALB/c mice. This may at least in part be the explanation for why little experimental work has been performed with *L. braziliensis* (Neal & Hale 1983, Childs et al. 1984). We recently reported (DeKrey et al. 1998) that following infection with *L. braziliensis* or *L. major*, BALB/c mice produced similar levels of IFN-g. However, *L. braziliensis* infected mice produced much less IL-4 (approximately 10-fold). In addition, when the *L. braziliensis* infected mice were treated with a neutralizing anti-IFN-g the animals were unable to resolve their infection. We concluded that BALB/c mice cure an infection with *L. braziliensis* because they produce a Th1 response that is unable to overcome the ability of IFN-g to activate *L. braziliensis* infected MØ's to kill the parasite.

Resolution of an infection with a particular species of *Leishmania* usually confers complete resistance to re-challenge with the same parasite. However, in addition to this, a primary infection with a given species of *Leishmania* can also confer cross-protection against a different species of *Leishmania* (Lainson & Bray 1966, Lainson & Shaw 1977, Alexander & Phillips 1978a,b, Perez

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shown in several different mammalian hosts; the protection sometimes acts in only one direction (Lainson & Shaw 1977), and in some cases the sex of the host influences the cross-protection seen (Alexander 1988).

Since L. braziliensis is unable to trigger a strong Th2 response in BALB/c mice, we hypothesized that following resolution of an infection with L. braziliensis, BALB/c mice might be at least partially protected against challenge with L. major. We report here that previous exposure to L. braziliensis can confer complete protection against a subsequent challenge with L. major and that this protection is dependent upon IFN-γ production.

MATERIALS AND METHODS

Mice and parasites - Young adult female mice were used in all experiments. BALB/c mice were obtained from either the National Cancer Institute (Bethesda, MD) or Jackson Laboratory (Bar Harbor, ME). C57BL/6 were obtained from the National Cancer Institute. Stationary phase promastigotes of L. braziliensis (MHOM-BR-79-LTB111) or L. major (RHO-SU-59-P) were used. Parasites were maintained as described (Titus et al. 1984).

Infecting mice and determining parasite numbers in cutaneous lesions - Mice were injected with the numbers of promastigotes indicated in the text in one hind footpad and lesion development was followed by measuring the thickness of the infected footpad compared to the thickness of the same footpad prior to infection.

Parasite numbers were determined in infected footpads using a published limiting dilution assay for determining parasite burdens in infected mouse tissues (Lima et al. 1997).

In some experiments mice were treated with a neutralizing anti-IFN-γ (XMG1.2) antibody as described in DeKrey et al. (1998).

Determining levels of cytokines in culture supernatants - At various times after infection, 3-5 mice per group were killed for evaluation. Single cell suspensions were prepared from draining lymph nodes (inguinal and popliteal). Cells were adjusted to 5×10^6/ml in Dulbecco's modified Eagle medium (Maryanski et al. 1982) containing 0.5% normal mouse serum (Harlan Bioproducts, Indianapolis, IN). Cultures were stimulated with 10^6 L. major promastigotes/ml and the supernatant of the cultures was harvested 72 hr later (a time determined to be optimal for the cytokines examined) for analysis.

Levels of IFN-γ and IL-4 in culture supernatants were determined by enzyme-linked immunosorbent assay (ELISA) using techniques published elsewhere (Soares et al. 1997).

Statistical analysis - Significance was determined using an non-paired t-test. Differences were considered to be significant when $p < 0.05$. All experiments shown are representative of two to three independent experiments.

RESULTS

To determine whether previous exposure to L. braziliensis led to protection against a subsequent challenge with L. major, we first experimented with the dose of L. braziliensis and the time between infection with L. braziliensis and challenge with L. major. We found that a large dose of L. braziliensis (10^7) administered subcutaneously (s.c.) in one hind footpad led to complete protection against a subsequent challenge with 10^6 L. major s.c. in the opposing hind footpad (Fig. 1). Moreover, the protective effect of pre-infecting with L. braziliensis was a dose titratable phenomenon. As shown in Fig. 1, a dose of 10^3 L. braziliensis led to the least protection against challenge with L. major whereas a dose of 10^7 L. braziliensis led to the greatest protection. Lesions of L. major were the largest in mice pre-treated with 10^3 L. braziliensis and only 20% of the mice (see numbers in the legend of Fig. 1) cured these L. major-induced lesions; in contrast, lesions of L. major were the smallest in mice pre-treated with 10^7 L. braziliensis and 100% of the mice cured these L. major-induced lesions.

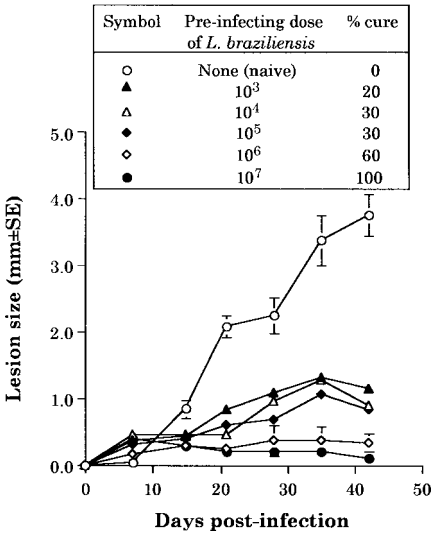

Fig. 1: course of infection with Leishmania major in BALB/c mice pre-infected with different concentrations of L. braziliensis. Groups of 10 BALB/c mice each were pre-infected with the indicated doses of L. braziliensis s.c. in one hind footpad. Twelve weeks later these animals were challenged s.c. in the opposing hind footpad with 10^6 L. major. Controls consisted of naive mice infected with 10^6 L. major. Lesions were monitored as described in Materials and Methods.

We also determined that the degree of resistance to challenge with L. major increased with time after exposure to L. braziliensis. Little if any protection against challenge with L. major was achieved when the two parasites were injected simultaneously. Some protection was observed when mice were challenged with L. major at 6 or 8 weeks after exposure to L. braziliensis. However, 100% protection against challenge with L. major was consistently achieved only at 12 weeks after exposure to L. braziliensis (data not shown). Importantly, at 12 weeks post-L. braziliensis injection, we were also unable to detect viable L. braziliensis in treated mice by limiting dilution analysis (data not shown). Therefore, for the remaining experiments presented here, mice were treated with 10^7 L. braziliensis and challenged 12 weeks later with 10^6 L. major.

The experiment shown in Fig. 1 demonstrated that pre-infection with L. braziliensis allows BALB/c mice to control the outgrowth of lesions of L. major when the mice were challenged with the parasite. To determine whether this was accompanied by destruction of L. major in the lesions, we measured the parasite burdens in the lesions. In L. braziliensis-naive control mice, L. major continued to replicate through day 42 of infection (Table I). In contrast, in mice pre-infected with L. braziliensis 12 weeks earlier, L. major was destroyed such that by day 42 of the experiment there were approximately 2,000-fold fewer parasites in their lesions compared to control mice (Table I).

We next analyzed the mechanism underlying the protection seen against challenge with major in BALB/c mice pre-infected with L. braziliensis. We first noted that an intense swelling response occurred in the footpads of L. braziliensis pre-treated mice when the mice were challenged with L. major (Fig. 2). This swelling response was characteristic of delayed-type hypersensitivity (DTH) in that it peaked from 24 to 48 hr post-challenge with L. major and it persisted to 72 hr post-challenge (Fig. 2). This observation suggested that cross reactive antigenic epitopes exist in L. braziliensis and L. major that prime T cell responses. Moreover, since DTH is mediated by Th1-type T cells (Mosmann & Coffman 1989), this also suggested that infection with L. braziliensis triggered Th1 T cells in BALB/c mice that could recognize L. major antigen(s) when the mice were challenged with the parasite.

To test the hypothesis that cross reactive Th1 T cells were elicited by pre-infection with L. braziliensis, we measured the cytokines produced when lymph node cells from L. braziliensis pre-infected mice were challenged with L. major in vitro. We first harvested the popliteal and inguinal nodes draining the footpad of mice pre-infected with L. braziliensis 12 weeks earlier. These cells were stimulated with L. major promastigotes in

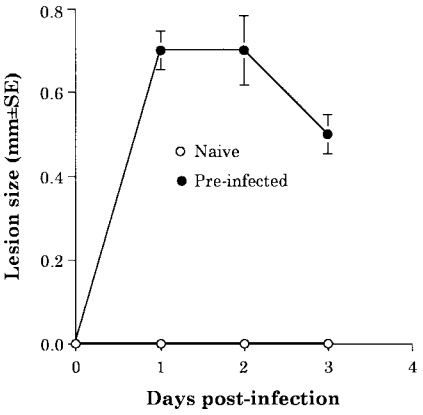

Fig. 2: footpad swelling response of Leishmania braziliensis pre-infected BALB/c mice challenged with L. major. BALB/c mice were pre-infected with L. braziliensis and challenged with L. major as described in the legend of Fig. 1.

TABLE I

Numbers of Leishmania major in lesions of BALB/c mice pre-infected with L. braziliensis

Days post-L. major infection	Number L. major/footpad lesion (95% confidence limits)	
	Naive	Pre-infected
3	0.24×10^5 (0.06-0.43)	0.04×10^5 (0.01-0.07)[a]
7	2.85×10^5 (1.06-4.63)	0.40×10^5 (0.10-0.71)
21	35.77×10^5 (9.63-61.90)	3.75×10^5 (1.40-6.05)
42	79.75×10^5 (23.70-135.80)	0.40×10^5 (0.01-0.08)

a: BALB/c mice were infected with 10^7 L. braziliensis s.c. in a hind footpad. Twelve weeks later, the mice were challenged in the opposing footpad with 10^6 L. major. Controls consisted of age-matched L. braziliensis-naive BALB/c mice challenged with 10^6 L. major. At the indicated time points after challenge, the footpad lesions from duplicate mice of each group were subjected to limiting dilution analysis to determine the number of L. major present.

vitro and the supernatants were harvested 72 hr later to determine their content of IFN-γ.