

Analysis of the Immune Responses of Mice to Infection with *L*

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supernatant, and *L. braziliensis* elicited 2.9 \pm 1.8 ng/ml. Finally, TNF- α production in response to infection with either species of parasite was not detected. This inability to detect TNF- α production was not due to technical failure, since C3H mice produced substantial levels of TNF- α following infection with *L. major* (e.g., 257.7 pg/ml of culture supernatant at day 3 postinfection).

IL-4 plays a central role in the susceptibility of BALB/c mice to infection with *L. major*. Therefore, since BALB/c mice heal an infection with *L. major*, we predicted that less IL-4 would be produced by these mice. Significantly (by analysis by nonpaired *t* test; a *P* value of < 0.05 was considered significant) less IL-4 (10- to 15-fold) was produced in response to infection with *L. major* than in response to infection with *L. braziliensis*, and by day 42 postinfection, *L. major*-infected mice did not produce detectable levels of IL-4 (Table 1). It should also be noted that when C3H mice are infected with *L. major*,

the mice develop barely perceptible cutaneous lesions and never make a detectable IL-4 response (data not shown).

Because IL-4 can inhibit the protective effects of IFN- γ in mice infected with *L. major* (7, 9), and because *L. major*-infected BALB/c mice produced significantly less IL-4 than *L. braziliensis*-infected BALB/c mice (Table 1), we hypothesized that the amount of IFN- γ produced by *L. major*-infected mice might be sufficient to control infection. Therefore, we treated *L. major*-infected mice with a neutralizing anti-IFN- γ .

First, we tested the potency of our neutralizing anti-IFN- γ preparation (anti-IFN- γ was purified from the ascites fluid of the anti-IFN- γ -producing hybridoma XMG1.2 [a gift from R. Coffman, DNAX, Palo Alto, Calif.] by salt precipitation [17, 18] by determining whether it would prevent C3H/HeJ mice (National Cancer Institute) from healing an infection with *L. major* as reported by others (3). Our anti-IFN- γ preparation converted C3H mice into animals completely susceptible to infection with *L. major* (see Fig. 1, inset). Since C3H mice produce considerably more IFN- γ than BALB/c mice following infection with *L. major* (16), and since BALB/c mice produce equivalent amounts of the cytokine following infection with either *L. major* or *L. braziliensis*, our preparation of anti-IFN- γ would be more than sufficient to neutralize IFN- γ in BALB/c mice infected with *L. major*.

Treating *L. major*-infected BALB/c mice with anti-IFN- γ (by intraperitoneal injections of 1.0 mg of XMG1.2 on the day of infection and at weekly intervals thereafter until the completion of the experiment) significantly enhanced lesion size and prevented the mice from resolving their infection (Fig. 1).



In addition, anti-IFN- γ treatment caused mice to produce more IL-4 in response to infection with *L. major*. For instance, at 2 weeks following infection, lymph node cells draining the lesion in treated mice produced 58.5 \pm 1.6 pg of IL-4/ml (mean \pm SD) when the cells were restimulated in culture with *L. major*. In contrast, untreated control mice produced 10.8 \pm 1.6 pg of IL-4/ml, or 5.4-fold less IL-4 (in cultures not restimulated with *L. major*), no IL-4 was detected). Finally, by day 144 of infection, anti-IFN- γ treatment had markedly enhanced parasite burden in the lesions (122 parasites/lesion in control mice versus 43.5 \times 10⁶ in treated mice, which is a 356,557-fold difference), which resulted in systemic infection with *L. major*, as evidenced by the fact that large numbers of the parasite (3.58 \times 10⁴) could be detected in the opposing (uninfected) footpad (Fig. 1). It is possible that parasites could be isolated from the opposing footpad because the *L. major* strain used (LTB-111) was originally isolated from a cutaneous lesion. Therefore, the parasite may prefer the lower temperature of cutaneous sites. Taken together, these data suggest that an IFN- γ -dependent mechanism is responsible for the killing of *L. major* by BALB/c mice.

IL-4 exacerbates disease in *L. major*-infected BALB/c mice (15). This predicts that neutralizing IL-4 in mice infected with *L. major* would lessen disease severity. Lesions on anti-IL-4-treated mice (for techniques, see reference 10) infected with *L. major* resolved in half the time and never were greater than 20% of the size of lesions on control mice (data not shown).

These data suggest that the weak infectivity of *L. major* for mice may be due to the inability of the parasite to elicit strong and sustained IL-4 production in the animals. Alternatively, it is possible that *L. major* is unable to elicit the production of other cytokines that inhibit the development of a Th1 response. IL-10 inhibits Th1 development; however, BALB/c mice produced equivalent amounts of IL-10 following infection with either *L. major* or *L. braziliensis* (see above). Transforming growth factor β (TGF- β) also inhibits Th1 responses. TGF- β correlates with susceptibility to infection with both *L. major* (2; reviewed in reference 13) and *L. braziliensis* (19). However, we have been unable to detect TGF- β (protein or mRNA, in vitro or in vivo) following infection with *L. major*. Since different isolates of *L. major* vary in their ability to induce TGF- β production (1), it is possible that LTB-111 is a poor inducer of TGF- β .

In conclusion, the data presented here extend the Th1 (protective)/Th2 (exacerbative) paradigm in leishmaniasis established by injection of different mouse strains with *L. major*. However, the approach taken here is unique. The Th1/Th2 paradigm with *L. major* was formulated by injecting different mouse strains with the parasite or by injecting the same mouse strain with the parasite followed by intervention with neutralizing anti-cytokines (anti-IL-4 or anti-IFN- γ) (reviewed in references 4, 8, 13, 14, and 21). Here, the same mouse strain (BALB/c) was injected with two leishmanial species that cause cutaneous disease; parasites that elicited a strong Th2 (IL-4) response survived (*L. major*), while those that did not (*L. braziliensis*) were killed.

This work was supported by NIH grant AI-29955. H.C.L. received

partial support from a World Health Organization TDR-WHO scholarship (M8/181/4/L.238).

The technical assistance of Monica Estay and Julie Bleyenbergh is gratefully acknowledged.

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