Cyclosporin A Treatment Converts *Leishmania* donovani-Infected C57BL/10 (Curing) Mice to a Noncuring Phenotype

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Survival of the amastigote form of *Leishmania* species in the phagolysosomal compartment of tissue macrophages is a hallmark of these protozoan pathogens (8). Murine models of disseminated leishmaniasis have provided fundamental information regarding the acquisition of immunity against *Leishmania major* and *L. donovani* (2, 3, 6, 8, 10). There is a large body of evidence showing that protective immunity requires effective cell-mediated responses while antibody responses to the parasites play no positive role (8, 10). A characteristic feature of disseminated leishmaniasis of humans and experimental animals is severe immune suppression involving cell-mediated responses. Although the mechanism of suppression is not fully understood, it is thought to be related to the appearance of suppressor T cells following amastigote parasitization of large numbers of tissue macrophages. The generation of suppressor T cells in experimental infections of mice can be blocked by cyclophosphamide (18), sublethal irradiation (2, 13), or adult thymectomy (6). These immunomodulating measures have successfully converted noncuring strains of mice to a curing phenotype.

A much studied model of disseminated leishmaniasis utilizes *L. major* and BALB/c mice challenged subcutaneously. Cutaneous lesions in this strain of mice progressively enlarge until the infection eventually becomes systemic (6). With this experimental model, two groups of investigators independently reported that multiple injections of cyclosporin A (CyA) prevented progression of cutaneous lesions and fatal visceralization (1, 15). CyA, a fungal metabolite, is used extensively in human organ transplantation because of its potent immunosuppressive properties. Since CyA does not exert a direct toxic effect on amastigotes, Solbach et al. (15) concluded that the suppressive effect of CyA on *L. major* infection in BALB/c mice was exerted indirectly by inhibiting normal function of T cells and macrophages which play a central role in the development of cutaneous lesions. T cells (Lyt-1⁺) appear to be the primary target of CyA (1, 10, 15); specifically, the drug is thought to inhibit activation of T lymphocytes and the release of interleukin-2, macrophage-activating factor, and gamma interferon (IFN-γ) (4, 5, 14). Thus, CyA suppression is attendant with a decreased inflammatory response (1) and inhibition of macrophage accumulation at the site of infection. Solbach et al. (15) speculated that a paucity of macrophages in the lesion limited the severity of *L. major* infection in CyA-treated BALB/c mice. These mice, however, did not develop curative immunity since the inhibitory effects of CyA on macrophage-activating factor and IFN-γ production prevented total elimination of *L. major* amastigotes within the residual macrophage population.

We considered it of interest to determine how CyA would modify the course of *L. donovani* infection in spontaneously curing C57BL/10 mice. Although this strain of mice is susceptible to *L. donovani* infection (Lsh), immunity and cure usually are achieved within several months (2, 12). Although both cutaneous (*L. major*) and disseminated (*L. donovani*) infections require effective cell-mediated responses for their resolution, it is now appreciated that, in susceptible murine hosts, the Lsh gene control of intrahost amastigote multiplication is operative only with *L. donovani* (3, 9). The course of *L. donovani* infection in CyA-treated C57BL/10 mice was followed so that the effects of the drug could be compared with the ameliorating effect of CyA on *L. major* infection in BALB/c mice (1, 15). Female C57BL/10 mice (Harlan, Indianapolis, Ind.) weighing 20 to 25 g were used throughout. *L. donovani* Sudan 1S amastigotes were obtained from spleens of infected hamsters as described previously (12). One hundred mice were challenged intravenously with 5 × 10⁶ amastigotes. CyA (Sandimmune, 50 mg/ml; Sandoz, East Hanover, N.J.) was diluted in phosphate-buffered saline. One group of 50 mice was injected intraperitoneally daily with 0.1 ml of CyA at a concentration of 50 mg/kg (1.25 mg/mouse) starting 1 day prior to challenge and continuing daily until the parasite burden was determined. The second, untreated group of mice served as infection controls. Groups of seven mice from each group were sacrificed on days 1, 10, 20, 30, 40, 50, and 60 postinfection. Livers and spleens were excised, and the number of parasites in each organ was determined by microscopic evaluation of stained (Diff-Quick; American

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Scientific Products, McGaw Park, Ill.) tissue imprints. Parasite burden was calculated by application of Stauber's formula (16); number of amastigotes/number of cell nuclei × weight of liver (milligrams) × 2 × 10^9 = total amastigotes per liver. CyA-treated C57BL/10 mice exhibited a continuous increase in severity of infection. At day 60 postchallenge, the liver parasite burden had attained a level of ca. 10^9 amastigotes (Fig. 1). Spleens also were heavily infected between days 30 and 60 of infection (data not shown). Untreated C57BL/10 mice exhibited a parasite burden of ca. 10^6 per liver at 20 days postchallenge, but predictably (2, 12) developed curative immunity over the next 30 days. Thus, a regimen of CyA similar to that which afforded protection of BALB/c mice against the development of systemic L. major infection was detrimental to C57BL/10 mice challenged with L. donovani by the intravenous route.

The contrasting effects of CyA on the course of the two infections may reflect fundamental differences in the pathogenesis of these experimental models of disseminated leishmaniasis. B10.D2 mice, a strain congenic with C57BL/10, is a noncuring phenotype and behaves similarly to the CyA-treated mice challenged with L. donovani. Ulczak and Blackwell (18) and Nickol and Bonventre (13) showed that sublethal irradiation of B10.D2 mice prior to challenge with L. donovani enabled the mice to develop curative immunity, i.e., converted B10.D2 to a curing phenotype. Howard et al. (6) had previously described a similar phenomenon with BALB/c mice sublethally irradiated before L. major challenge subcutaneously. In both cases, it was postulated that suppressor T cells and/or their precursors were eliminated by irradiation, thereby permitting positive T-cell signals and activation of the macrophage system. In the case of cutaneous leishmaniasis, the absence of T-cell-derived lymphokines suppressed the influx of potential host cells and the development of the leishmanial ulcer (1, 6, 15). In the L. donovani experimental model, however, infection is initiated primarily by parasitization of liver and spleen where an unlimited number of potential host cells, i.e., tissue macrophages, reside. Thus, by inhibiting the production of macrophage-activating factor and IFN-γ, CyA exacerbated L. donovani infections of C57BL/10 mice. It is accepted that acquisition of protective immunity in leishmaniasis depends on effective signaling between specifically sensitized T cells and macrophages (8, 10). T cells from C57BL/10 mice infected with L. donovani cease responding to concanavalin A and leishmanial antigens as the parasite burden becomes significant (12, 13). This immunosuppressive phase of infection is normally followed by restoration of T-cell responses and is coincident with the elimination of amastigotes from infected macrophages. Recovery is mediated by T-cell products such as interleukin-2 and IFN-γ (11). CyA treatment of L. donovani-infected C57BL/10 mice, however, by depressing helper T cells of the Lyt-1+ phenotype prevented the onset of the immune recovery phase. A similar immunosuppressive effect of CyA has been demonstrated in another T-cell-dependent infection model (7), Mycobacterium bovis BCG infection of BALB/c mice (17). Excavation of systemic BCG infection was also attributed to inhibition of effector T cells and production of IFN-γ.

In the L. major model, CyA may exert a selective suppression of parasite-specific TH1 (disease-enhancing) cells, thereby favoring expansion of the TH2 (protective) cell population (10). Alternatively, the drug may stabilize the absolute number of T helper cells at a level favoring containment rather than exacerbation of the cutaneous infection (10). There is no evidence, however, that selective appearance of TH1 or TH2 lymphocytes occurs in L. donovani disseminated infection. Precise mechanisms of CyA action in both models of leishmaniasis remain conjectural. Our data reinforce, however, the crucial role played by the T-cell arm of the immune response against L. donovani. Also, the completely opposite effects of CyA on the course of L. major and L. donovani infections in these two widely used experimental models of disseminated leishmaniasis can be reconciled by differences in the routes of infection and the tissue distribution and availability of macrophages which serve as obligatory host cells for both protozoan species.

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LITERATURE CITED


