Models of Relapse of Experimental Visceral Leishmaniasis

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To establish models for studying recurrence of visceral leishmaniasis, a growing problem in T cell–deficient patients, two approaches were investigated: treatment of euthymic BALB/c mice with quiescent *Leishmania donovani* infection with T cell–depleting or anti-cytokine antibodies and serial observation of acutely infected nude BALB/c mice after an initial antileishmanial response induced by amphotericin B treatment. In chronically infected euthymic mice, maintenance of acquired immunity and prevention of relapse required CD4 cells and a multicytokine-dependent mechanism involving endogenous interleukin-2, interferon-γ, and tumor necrosis factor-α. Acutely infected nude mice responded to amphotericin B with a >85% reduction in liver parasite burdens; however, after a brief lag, visceral infection readily recurred in the posttreatment period. Both models may be useful for testing experimental interventions designed to reduce relapse of previously controlled visceral leishmaniasis in T cell–deficient hosts.

Despite being rendered clinically quiescent by drug therapy or a spontaneous immune response [1,2], visceral leishmaniasis (kala-azar) is nevertheless prone to relapse. Increasing parasite drug resistance or suboptimal treatment regimens (or both) likely contribute to relapse in otherwise healthy persons [3]. In areas in which the disease is endemic, however, recrudescence is also now recognized as a growing clinical problem in 2 T cell–impaired populations: CD4 cell–deficient patients with advanced human immunodeficiency virus (HIV) infection [4] and patients treated with immunosuppressive agents [5]. Relapse in such persons is not surprising because successful host defense in visceral leishmaniasis is T cell–dependent [6,7] and because visceral parasites are probably seldomly eliminated entirely by either drug treatment or the immunologic mechanisms of spontaneously acquired resistance [7].

To experimentally investigate relapse of kala-azar in T cell–deficient hosts, we have developed two models in *Leishmania donovani*–infected animals. One model uses chronically infected euthymic mice subjected to depletion of T cells or relevant cytokines and the other uses T cell–deficient nude mice that have responded to prior chemotherapy. Both models should be helpful in future testing of experimental interventions designed to reduce relapse of kala-azar in T cell–impaired patients.

Materials and Methods

Visceral infection. Female euthymic BALB/c mice (Charles River Breeding Laboratories, Wilmington, MA) and athymic (nude) BALB/c mice (Life Sciences, St. Petersburg, FL) weighing 20–30 g were infected via the tail vein with 1.5 × 10⁷ *L. donovani* amastigotes as described [8]. At the indicated time points, visceral infection was assessed in liver imprints by microscopic counting; liver parasite burdens are expressed as Leishman-Donovan units (LDU) [8]. Data analysis was done using Kruskal-Wallis one-way analysis on ranks; Dunn’s method was used for multiple comparisons [9].

Treatment of euthymic mice with T cell–depleting or anti-cytokine antibodies. Euthymic mice, which reduce visceral burdens by 80%–90% within 8 weeks [8], were left undisturbed for 6 months after infection. After that length of time and for 6–12 additional months, euthymic mice remain healthy, show low levels of persistent visceral infection (<100 LDU), and, as judged by resistance to rechallenge, remain solidly immune to *L. donovani* [8].

Six months after infection, immune mice were sacrificed to determine baseline liver burdens. Groups of 3 to 4 mice were injected intraperitoneally (ip) for 8 weeks using previously described antibody preparations and administration schedules [6,8–10] (see legend to figure 1) that inhibit acquired resistance in naive mice [6,9,10] and resistance to rechallenge in immune mice [8]. The preparations included 1 mL of hybridoma culture supernatants containing rat anti-mouse monoclonal antibodies (MAbs) GK 1.5 (anti-CD4, ATCC TIB 207, 8 μg/mL IgG), 53–67.2 (anti-CD8, ATCC TIB 105, 12 μg/mL IgG), and S4B6.1 (anti-interleukin [IL]-2, 12.5 μg/mL IgG) or 25 μg of normal rat IgG (Sigma, St. Louis) suspended in saline. They also included 0.2 mL of normal rabbit serum, rabbit anti-mouse tumor necrosis factor-α (TNF-α) antisemur (1.5 × 10⁵ neutralizing U/mL), or rabbit anti-mouse interferon-γ (IFN-γ) antisemur (10⁵ neutralizing U/mL) [6,8–10]. Fluorescence analysis of spleen cells [6] from mice treated with anti-CD4 or anti-CD8 revealed 89%–92% depletion of the targeted T cell subset at the end of the first week of injections; comparable depletion (>90%) was maintained at week 8 by twice-weekly MAb injections (data not shown).

Treatment of nude mice with amphotericin B. Two weeks after infection, liver parasite burdens were determined, and groups of 3 nude mice were treated ip with 5 mg/kg/day of amphotericin B (Fungizone; E. R. Squibb & Sons, Princeton, NJ) every second
day for a total of three injections [11]. Parasite burdens were measured 2 days, 3 weeks, and 12 weeks after treatment. Euthymic naive BALB/c mice were treated identically.

Results

Relapse of chronic quiescent infection in euthymic mice.

Following an initial 4-week period during which visceral *L. donovani* freely replicates, naive euthymic BALB/c mice acquire resistance and proceed to reduce liver and spleen parasite burdens [8]. Thereafter, these mice remain persistently infected at low levels for an indefinite period [8]. These animals resist rechallenge, and their spleen cells respond to specific antigen with IL-2 and IFN-γ secretion and transfer resistance to naive mice [8]. As judged by the effects of MAb treatment (figure 1), maintaining intracellular *L. donovani* in a quiescent state and prevention of reactivation of visceral infection required CD4 cells and appeared to involve a multicytokine-dependent endogenous mechanism. CD8 cells may also play a role, since although the effect induced by anti-CD8 treatment was not significant (*P* > .05), liver burdens were nevertheless increased 2-fold in CD8 cell–depleted mice. The results shown in figure 1 also suggest that IL-2, IFN-γ, and TNF-α each participated in this mechanism. After 8 weeks of anti-cytokine antibody administration, liver parasite burdens were increased 5.0-, 8.3-, and 6.5-fold, respectively, over burdens in mice injected with control preparations.

Posttreatment relapse in nude mice. Nude BALB/c mice, which fail to control *L. donovani* visceral replication [6, 9–11], were selected as a model with which to examine relapse after treatment in a T cell–deficient host. While these mice do not respond to conventional antimony unless reconstituted with T cells [11, 12], they do respond normally to amphotericin B [11], a potent leishmanicidal agent with increasing clinical use [13, 14]. To test the hypothesis that posttreatment relapse would likely develop in the absence of T cells, infected nude mice were first treated with amphotericin B for 1 week and then left undisturbed for up to 12 additional weeks. As shown in figure 2, liver parasite burdens in euthymic mice continued to decline after treatment; the opposite result was observed in nude mice. Despite the comparable initial response to amphotericin B, liver burdens in nude animals doubled 3 weeks after treatment and then steadily increased (figure 2).

Discussion

Together, these results reemphasize the critical role of T cells (and T cell–derived or –regulated cytokines) in preventing spontaneous relapse of previously controlled, quiescent infection and in maintaining the posttreatment response. In euthymic mice with chronic but well-suppressed infection, a multicytokine-dependent mechanism appears to be involved and may be similar to the endogenous response that initiates acquisition of resistance to *L. donovani* in naive animals. In this latter model, IL-2 induces IFN-γ, and IFN-γ and TNF-α appear to be linked factors in a more distal antileishmanial effector pathway [9]. Thus, the results in figure 1 suggest that the maintenance of
immunity (prevention of relapse) in chronic *L. donovani* infection likely involves the same sequential type of cytokine-mediated mechanism as well. It is possible that IL-12 [10] may also participate as a more proximal endogenous component of this same mechanism.

Since new treatment strategies are needed to address the issue of relapse in T cell–deficient patients with visceral leishmaniasis, both of the models described here may prove useful in testing interventions directed at suppressing reemergence of infection. For example, and consistent with our results in amphotericin B–treated mice, HIV-infected patients with kala-azar also show good initial responses to treatment with lipid formulations of amphotericin B but mostly relapse once therapy is discontinued [14]. Whether this outcome can be successfully altered (e.g., by the addition of long-term cytokine treatment) is not known. IFN-γ would seem appropriate to test experimentally, as judged by the capacity of anti–IFN-γ treatment to provoke relapse (figure 1) and in view of its already recognized efficacy in both experimental and human kala-azar [15]. CD4 cell–depleted, chronically infected euthymic mice also represent another experimental setting in which it would be logical to test the capacity of exogenous IFN-γ or inducers of endogenous IFN-γ (IL-2 and IL-12 [9, 10]) to prevent relapse.

References