Prevention of Relapse after Chemotherapy in a Chronic Intracellular Infection: Mechanisms in Experimental Visceral Leishmaniasis

Henry W. Murray

http://www.jimmunol.org/content/174/8/4916

References
This article cites 81 articles, 40 of which you can access for free at:
http://www.jimmunol.org/content/174/8/4916.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Prevention of Relapse after Chemotherapy in a Chronic Intracellular Infection: Mechanisms in Experimental Visceral Leishmaniasis¹

Henry W. Murray²

In visceral leishmaniasis, chemotherapy probably seldom eradicates all parasites in tissue macrophages; nevertheless, most T cell-intact patients show long-lasting clinical cure after treatment despite residual intracellular infection. To characterize prevention of posttreatment relapse, amphotericin B was used to kill ~90–95% of Leishmania donovani in livers of mice deficient in mechanisms of acquired antileishmanial resistance. Recrudescence subsequently developed 1) in animals deficient in both CD4 and CD8 T cells as well as CD40L-mediated T cell costimulation, but not in a) CD4 or CD8 cells alone, b) NK cell lytic activity, or c) ICAM-1-recruited monocytes; and 2) in mice deficient in IFN-γ, but not in the IFN-γ-inducing cytokines, a) IL-12, b) IL-12 and IL-23, or c) IL-18. Posttreatment recrudescence also did not develop in animals deficient in macrophage phagocyte NADPH oxidase (phox) or inducible NO synthase (iNOS) alone or, surprisingly, in those deficient in both phox and iNOS. Therefore, regulation of the intracellular replication of residual Leishmania donovani that escape chemotherapy evolves to a host mechanism distinguishable from initial acquired resistance at the T cell, cytokine, and macrophage levels. Posttreatment, either CD8 or CD4 cells can direct the response, IL-12 is not required, and iNOS and phox, the activated macrophage’s primary IFN-γ-inducible leishmanicial pathways, both become dispensable. The Journal of Immunology, 2005, 174: 4916–4923.

Visceral leishmaniasis (kala-azar) is a disseminated intracellular protozoal infection that targets tissue macrophages in the liver, spleen, and bone marrow. In otherwise immunocompetent patients who develop fully expressed disease, treatment with pentavalent antimony (Sb),³ amphotericin B (AmB), or miltefosine induces initial clinical and parasitologic responses in >90% of cases (1, 2).³ However, relapse after an apparent response to any drug regimen is well recognized in kala-azar, even in phenotypically normal individuals. The risk of recurrence is highest in the first 6–12 mo after therapy (1–5); thus, treated patients are not considered complete clinical responders (definitive cures) until at least 6 posttreatment months have passed uneventfully (6).

However, neither chemotherapy (nor a satisfactorily expressed T cell-dependent host immune response (1)) is thought to eradicate all tissue parasites in any form of leishmaniasis (7–18). In successfully treated kala-azar, for example, visceral macrophages are presumed to harbor quiescent parasites, probably for life. A similar state of chronic intracellular parasitism apparently exists in a considerably larger population as well, individuals also infected with visceralizing Leishmania species, but without expression of clinical illness (2, 19, 20). In either setting, T cell-dependent effects along with cytokine-regulated macrophage activation probably maintain treated and subclinical infection in the latent state (1, 2, 9–14, 16, 18, 20–24). Although residual tissue parasites presumably provide ongoing immunization important in resistance to re-infection (18, 25, 26), the same organisms also represent an endogenous reservoir for potential recrudescence of symptomatic kala-azar. Such a reservoir places individuals previously infected with viscerotropic Leishmania, including patients properly treated, at some risk for future relapse. Clinically, this risk clearly increases if T cell (CD4 cell)-dependent mechanisms become impaired (2, 16, 22, 24).

Experimental studies in mouse models of visceral infection, including those using Leishmania donovani in initially susceptible, but self-curing wild-type (WT) BALB/c and C57BL/6 mice, have identified acquired host responses that 1) induce initial resistance (1, 2, 27–40), 2) maintain immunity and prevent spontaneous relapse (11, 12), and 3) impart solid resistance to rechallenge (10). These three basic responses are T cell-dependent; involve an array of cytokines including IL-12, IFN-γ, and TNF; and are expressed in the tissues by granulomatous inflammation and macrophage activation (1, 2, 27–42). However, depending upon the acquired antileishmanial response tested and the model of visceral infection used, specific requirements for sensitized CD4 and/or CD8 cells and individual activating cytokines may vary (10, 11, 27, 39, 42, 43). Thus, it seemed reasonable to consider that prevention of relapse of chronic infection after antileishmanial chemotherapy might reflect distinct host defense mechanisms as well.

Prior experimental and clinical observations have made it clear that the protective posttreatment mechanism in visceral infection requires T cells (2, 5, 11, 16, 22, 44). To learn more about this response, which bears conversely on how relapse may occur in treated kala-azar, we tested outcome after chemotherapy in L. donovani-infected mice deficient in a spectrum of antileishmanial...
mechanisms. AmB was used in this study because, unlike the experimental effect of Sh, AmB’s leishmanicidal activity is direct and fully expressed initially in both T cell- and cytokine-deficient animals (1, 31, 34–37, 40, 45, 46).

**Materials and Methods**

**Mice**

Twenty to 30 gram female C57BL/6 and BALB/c mice purchased from The Jackson Laboratory were used as controls. Twenty to 30 gram female athymic (nude), CD4-/-, CD8-/-, and bg/bg (beige) mice on a C57BL/6 background were also purchased from The Jackson Laboratory. Breeding pairs of gene-disrupted (knockout (KO)) mice on a C57BL/6 background were originally obtained from the following sources: CD40L-/-, ICAM-1-deficient, and IFN-γ-/- (The Jackson Laboratory); respiratory burst (phagocyte NADPH oxidase (phox))-deficient g9pHPOS-/-/ (double KO), Dr. C. Nathan (Weill Medical College, New York, NY) (35); and IL-18-/-, Dr. C. Biron (Brown University School of Medicine, Providence, RI) (48). Breeding pairs of IFN-γ-/-, IL-12p55-/-, and IL-12p40-/- mice on a BALB/c background were obtained from The Jackson Laboratory. Male and female KO mice were used in a random fashion, and bred mice were 6–13 wk old when challenged with *L. donovani*. These studies were reviewed and approved by the institutional animal care and use committee of Weill Medical College.

**Visceral infection and tissue response**

Groups of three to five mice were injected via the tail vein with 1.5 x 10⁷ hamster spleen-derived *L. donovani* amastigotes (1 Sudan strain) (40). Visceral infection was followed microscopically using Giemsa-stained liver imprints in which liver parasite burdens were measured by blinded counting of the number of amastigotes per 500 cell nuclei imprints in which liver parasite burdens were measured by blinded counting of the number of amastigotes per 500 cell nuclei (42). The histologic response to infection was assessed microscopically in liver sections stained with H&E (42).

**Response to AmB treatment**

Starting 2 wk after infection, mice received three alternate-day i.p. injections of AmB (Gensia Laboratories), given at an optimal dose (5 mg/kg) on days 14, 16, and 18 (45). Liver parasite burdens were determined 14 and 21 days and 9 wk after treatment (12 wk after infection). Initial killing of the number of amastigotes per 500 cell nuclei imprints in which liver parasite burdens were measured by blinded counting of the number of amastigotes per 500 cell nuclei (42).

**Results**

**Posttreatment suppression of infection requires T cells**

In initially susceptible WT C57BL/6 mice with established *L. donovani* infection, AmB injections on days 14, 16, and 18 reduced liver parasite burdens by 91% on day 21 (Fig. 1A). Nine weeks later, parasite burdens in treated animals remained low at levels similar to those in untreated C57BL/6 controls at wk 12 (53 ± 12 LDU; two experiments; n = 8 mice). (The latter, like WT BALB/c mice, acquire resistance by wk 4 and spontaneously show near-resolution of *L. donovani* hepatic infection by wk 8 (27, 40)). In wk 12 liver sections from treated C57BL/6 mice, intracellular amastigotes were scarce, and tissue inflammation, expressed earlier during wk 2–4 as epithelioid granulomas (not shown) (40), had largely involuted (Fig. 2A).

**Response to AmB in CD4 and CD8 cell-deficient mice**

CD4 and CD8 KO mice were next tested in parallel with WT and nude animals to determine whether one T cell subset primarily acts to prevent relapse after chemotherapy. Consistent with findings in WT mice depleted of CD4 or CD8 cells by mAb injections (27), untreated CD4 and CD8 KO s failed to control *L. donovani* replication. At wk 12, liver burdens were high (3103 ± 291 and 3610 ± 315 LDU, respectively, vs 98 ± 16 LDU in WT mice; two experiments; n = 8–12 mice/group), and granuloma assembly was impaired (not shown). In response to AmB given during wk 3, parasite killing in CD4 and CD8 KO mice was comparable and similar to that in nude mice (88–93% liver parasite killing by day 21; Fig. 1B). Thereafter, however, posttreatment outcome in these three groups of mice clearly diverged, because infection did not recur in livers of CD4- or CD8-deficient mice. This result indicated that either CD4 or CD8 cells could provide the required T cell mechanism. The wk 12 liver histologic appearance in treated CD4 and CD8 KO mice was also similar (Fig. 2, C and D), showing evidence of residual inflammation and few visible amastigotes.

**NK cell-deficient mice**

Recurrent infection in treated nude mice indicated that NK cells retained in these animals were not sufficient to suppress parasite replication. However, NK cells may help to control *L. donovani* in T cell-intact mice, albeit in a limited fashion (10, 49). Therefore, euthymic beige mice, deficient in NK cell lytic activity (10), were also treated on days 14, 16, and 18 after infection. In these mice, AmB induced 94% killing by day 21 (two experiments; not shown), and liver parasite burdens in treated as well as untreated beige mice were low at wk 12 (88 ± 21 and 136 ± 14 LDU, respectively; n = 7–9 mice/group). Thus, NK cell lytic activity did not appear to contribute to posttreatment parasite suppression.
Response in CD40L-deficient mice

CD40:CD40L T cell costimulation, which can be expressed in CD4 and CD8 cells (50), regulates antileishmanial defense by optimizing IL-12 production, shaping the Th1 cell-type response, and inducing macrophage activation via T cell secretion of IFN-\(\gamma\) (40, 51, 52). Not surprisingly, CD40L KO mice fail to control \(L.\) donovani replication at wk 8 (40), and in two new experiments extended in this study to wk 12, liver parasite burdens reached remarkably high levels (7,636 ± 909 LDU; three experiments; \(n = 10\) mice). Although these mice are AmB responsive (40) and showed 93% parasite killing on day 21 (Fig. 1C), relapse seemed assured in the absence of CD40L once the drug effect had waned. This prediction was borne out by the >20-fold increase in parasite burdens between wk 3 and 12 in treated CD40L KOs (Fig. 1C). Infection recurred despite granulomatous responses at the majority of parasitized liver foci (Fig. 2E), indicating, as in untreated CD40L KO mice (40), that CD40L is required for control of \(L.\) donovani, but not for late-stage granuloma assembly.

Posttreatment recurrence in cytokine-deficient mice

**IFN-\(\gamma\) and IL-12.** Multiple cytokines participate in initial as well as memory responses in \(L.\) donovani infection (1, 10, 11, 28–34, 37, 39). Along with IL-12, for example, IFN-\(\gamma\) and TNF interdigitate prominently in macrophage activation and acquired resistance; if one of the three is deficient, visceral infection does not come under control (1, 31–34, 37, 53). Therefore, although AmB retains its initial leishmanicidal activity in the absence of IL-12, IFN-\(\gamma\), or TNF (31, 34, 37), outgrowth of surviving parasites would be anticipated in animals lacking these cytokines. This expectation was recently confirmed in TNF KO mice (37).

The experiments in Figs. 3 and 4 in BALB/c KO mice extend this posttreatment analysis to IFN-\(\gamma\) and IL-12. In untreated IFN-\(\gamma\) and IL-12p35 KO mice, liver parasite burdens at wk 12 were high (5,843 ± 621 and 3,825 ± 222 LDU, respectively; two or three experiments; \(n = 9–12\) mice), far exceeding wk 12 values in untreated WT BALB/c controls (81 ± 22 LDU; two experiments; \(n = 9\) mice). Although both groups of cytokine-deficient mice also showed similar initial responses to AmB (91–98% killing by day 21; Fig. 3A), parasite burdens and histologic findings were strikingly different at wk 12 (Fig. 3A and Fig. 4, A–D). IFN-\(\gamma\) KO mice
obviously relapsed after therapy, whereas IL-12p35 KOs did not, indicating posttreatment evolution to an IL-12-independent mechanism. The requirement for IFN-γ, also demonstrated in IFN-γ-deficient C57BL/6 mice (Fig. 3A), was underscored by reconstituting C57BL/6 nude mice with spleen cells from WT vs IFN-γ KO animals (Fig. 5). In contrast to WT spleen cells, which enabled suppression of parasite replication after AmB, transfer of IFN-γ-deficient cells provided little protective posttreatment effect.

IL-18 and IL-23. IL-12 is the primary IFN-γ-inducing cytokine in this L. donovani model (31–33). However, the preceding wk 12 observations pointed to an effect of other IFN-γ-inducing cytokines and prompted testing roles for IL-18 and IL-23 (48, 54, 55). At both wk 2 and 4, liver parasite burdens were significantly higher in untreated C57BL/6 IL-18 KO mice vs WT animals; however, KO mice spontaneously controlled infection by wk 8 (two experiments; data not shown). At wk 12, liver burdens remained low in untreated IL-18 KO mice (164 ± 32 LDU) and were still lower in IL-18 KOs given AmB during wk 3 (24 ± 7 LDU; n = 8 mice/group).

To gauge the contribution of IL-23, BALB/c IL-12p40 KO mice (deficient in both IL-12 and IL-23) (38, 54–56) were tested in parallel with IL-12p35 KO mice (deficient in IL-12 alone) (31). Untreated IL-12p40−/− mice did not control L. donovani replication at wk 12 (6012 ± 649 LDU; two experiments; n = 9 mice). However, these KO animals responded to AmB (89% killing on day 21) and, like p35-deficient mice, did not show recurrent visceral infection at wk 12 (Fig. 3B and Fig. 4, E and F). Thus, by itself and akin to IL-18, IL-23 was not apparently required to prevent relapse after therapy, leaving open the question of what late-acting compensatory mechanism induces IL-12-independent IFN-γ in the posttreatment state.
Figure 6. Photomicrographs of liver sections from mice deficient in macrophage leishmanicidal mechanisms 12 wk after infection. A, Untreated phox KO mice have largely cleared infection, whereas untreated iNOS KO (B) and iNOS/phox double-KO mice (C) show heavily parasitized Kupffer cells (arrows) surrounded by intense, but ineffective, granulomatus inflammation. D, Double-KO mice treated 9 wk previously with AmB show only scattered residual granulomas and no recurrent infection. Original magnification, ×200.

Response to AmB treatment, initial leishmanicidal activity was similar in the three groups of deficient animals (97–98% killing; Fig. 7). At wk 12, infection did not recur in the absence of either phox or iNOS alone, as anticipated (34), but remarkably, also did not recur in mice lacking both pathways (Figs. 6D and 7). Thus, at the level of the parasitized macrophage, neither of this effector cell’s primary antileishmanial mechanisms was required to prevent relapse of chronic infection in the liver after chemotherapy.

Discussion

In the basic expressions of acquired resistance in this model of experimental visceral leishmaniasis, acquisition of initial resistance, resistance to reinfection, and maintenance of immunity after spontaneous self-cure (1, 10, 11, 27–42, 57), T cells, cytokines, and activated macrophages interdigitate as recognized cornerstones of host defense. However, in this spectrum of acquired responses, distinguishing features can be identified, including differing requirements for participation of particular T cells (e.g., CD4,
CD8, or both subsets) (10, 11, 27) and/or cytokines (e.g., IFN-γ is not required in resistance to rechallenge) (10). Similarly, regulation of the posttreatment response tested in this study also showed separate T cell and cytokine requirements compared with those, for example, of acquired resistance (Table I). Together, these observations underscore 1) the general flexibility of host antileishmanial programs, including the likely presence of multiple compensatory pathways, and 2) specific modification (evolution) of mechanisms in response to varying experimental conditions. Examples of the latter include the stage of visceral infection examined (acute vs chronic), the use of intentional rechallenge, and whether conversion to the chronic state develops spontaneously or is induced by chemotherapy as in the present study (10, 11, 27, 42).

Table I, which summarizes mechanisms in this model of initial acquired resistance vs prevention of recurrence after chemotherapy, should be viewed with some caution, however. First, different stages of infection were analyzed, and different experimental hosts were used (e.g., C57BL/6 and BALB/c mice and mAb-treated WT and KO mice). Second, different results might have been generated if chronically infected WT mice had been injected after treatment with cell-depleting or cytokine-neutralizing mAb or treated with an iNOS inhibitor (13), rather than studying genetically deficient KO mice. Third, the latter may express responses or mechanisms not ordinarily observed in or used in the same fashion by normal animals (37). Nevertheless, testing in cell-, cytokine-, or enzyme-deficient KO animals can readily demonstrate the presence of such compensatory-type mechanisms as well as identify dispensable factors. Fourth, the initial parasite-lowering effect of treatment should not be discounted because it may have provided the opportunity for alternative host immune responses to develop and be expressed during the posttreatment period. In addition, because responses to *L. donovani* can be organ specific (32), results for infection in the spleen or bone marrow in KO mice might also differ from those observed in this study.

To maintain the intracellular *L. donovani* amastigotes that escape chemotherapy in a long-term quiescent state, the chronically infected host may also have more than one basic mechanism upon which to draw. For example, humoral factors might play some protective role, although relapse in human infection occurs despite the presence of specific Ab (2). Much more likely, then, is an intracellular response, such as characterized in this report, which proved to be T cell-dependent and cytokine regulated, although independent of the activated macrophage’s primary leishmanicidal mechanisms.

In these experiments, prevention of posttreatment recurrence 1) was directed equally well by CD4 or CD8 T cells, 2) involved CD40L signaling, and 3) required IFN-γ (and TNF (37)), presumably to maintain a certain level of macrophage activation. Although CD4 cells probably exert multiple effects (27, 59), the role of CD8 cells in macrophage activation in this *L. donovani* model has been less well appreciated, albeit previously noted (27, 60). Nevertheless, like CD4 cells, CD8 cells express CD40L and secrete IFN-γ, TNF, and a variety of chemokines (27, 50, 60, 61). Thus, the effects of both cytokines, possibly mediated by CD40L:CD40 signaling (62), may underlie the capacity of either T cell subset to successfully orchestrate protective posttreatment responses. Antileishmanial effects of CD8 cells might also relate to separate pathways, including, perhaps, the granzyme-perforin mechanism (60, 61, 63).

Our results in cytokine-deficient mice suggest that although IFN-γ (present report) and TNF (37) probably act in concert after treatment to suppress the replication of residual intracellular liver parasites, this effect does not require IL-12, the primary IFN-γ-inducing cytokine in this model (31). IL-23 and IL-18 also induce or enhance IFN-γ secretion (48, 54, 55); however, neither cytokine was by itself required to prevent posttreatment relapse. These observations may well reflect an alternative IFN-γ-producing pathway, apparently dependent on TNF, but independent of IL-12, IL-23, and IL-18, recently described in IL-12p40−/−/IL-18−−/− double-KO mice infected with *Mycobacterium tuberculosis* (55). Other IFN-γ-inducing stimuli, IL-2 (28), IL-15 (64), IL-27 (65), or

---

Table I. *Initial vs posttreatment acquired immune responses in L. donovani infection in the liver*

<table>
<thead>
<tr>
<th>Antileishmanial Factor or Mechanism</th>
<th>Acquisition of Initial Resistance</th>
<th>Prevention of Posttreatment Relapse KO Mice (present report)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cells</td>
<td>NT</td>
<td>Required†</td>
</tr>
<tr>
<td>CD4 cells</td>
<td>Required</td>
<td>Required†</td>
</tr>
<tr>
<td>CD8 cells</td>
<td>Required</td>
<td>Required†</td>
</tr>
<tr>
<td>CD40L</td>
<td>NT</td>
<td>Required†</td>
</tr>
<tr>
<td>NK cells</td>
<td>Not required</td>
<td>Required†</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Participates</td>
<td>Required†</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Participates</td>
<td>Required†</td>
</tr>
<tr>
<td>IL-2</td>
<td>Participates</td>
<td>Required†</td>
</tr>
<tr>
<td>IL-12</td>
<td>Participates</td>
<td>Required†</td>
</tr>
<tr>
<td>IL-23 (and IL-12)</td>
<td>NT</td>
<td>Required†</td>
</tr>
<tr>
<td>IL-18</td>
<td>NT</td>
<td>Participates</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Participates</td>
<td>NT</td>
</tr>
<tr>
<td>TNF</td>
<td>Required</td>
<td>Required†</td>
</tr>
<tr>
<td>iNOS</td>
<td>Participates</td>
<td>Required†</td>
</tr>
<tr>
<td>phox</td>
<td>NT</td>
<td>Participates</td>
</tr>
</tbody>
</table>

*Results are from prior reports from this laboratory (10, 27, 34 – 37, 40, 53, 57, 78 – 80), except for CD4, CD8, IL-12p40 (IL-23/IL-12-deficient), IL-18 and iNOS/phox double KO mice (present report). “Required” indicates no effective control of liver infection by 8–12 wk after challenge in mice deficient in the indicated factor. “Participates” indicates enhanced susceptibility early on (at wk 2–4) but either (1) evidence of control by wk 4–8 (reduced liver burdens, resolution of infection), or (2) for IL-12 and iNOS in WT mice, no data available after wk 4.*

*WT BALB/c and/or C57BL/6 mice treated before and/or after *L. donovani* challenge with appropriate cell-depleting or cytokine-neutralizing mAb or inhibitor (aminoguanidine for iNOS (34)). Note that WT BALB/c and C57BL/6 mice show similar susceptibility to *L. donovani* (wk 2–4) and both then self-cure (wk 4–8) without treatment (27,40).*
possibly IL-21 (66), may also be worth investigating in the chronically infected host in this posttreatment model.

Because *L. donovani* resides within tissue macrophages in all stages of visceral infection, the logical target and role for IFN-γ and TNF in maintaining a quiescent posttreatment state is the parasitized macrophage and its activation. Our results in livers of mice doubly-deficient in phox and iNOS demonstrate, however, that neither of these leishmanicidal pathways is required to prevent posttreatment recurrence. Because prevention of relapse requires inhibition of replication, but not necessarily intracellular parasite killing, it is possible that in this particular setting, IFN-γ and TNF evolve to regulate a leishmanastatic, rather than a leishmanicidal, mechanism. Either way, the results in double-knockout mice clearly uncover the presence and activity of a novel, compensatory antileishmanial mechanism not induced by *L. donovani* alone, but in this case triggered by AmB. Whether any form of effective antileishmanial chemotherapy can induce this macrophage mechanism (see below) will be clarified by testing Sb and/or miltefosine in iNOS/phox double-knockout mice. These same animals also show spontaneous macrophage antibacterial activity (47). Although the basis of this latter effect in doubly-deficient mice has not yet been identified, p47 GTPases have recently been implicated in a separate IFN-γ-inducible, iNOS- and phox-independent, macrophage antimicrobial mechanism (67, 68). This mechanism, which is active against intracellular pathogens (68), might also operate in *L. donovani* infection.

Although not investigated in this study, default to a suppressive-type cytokine response, mediated by IL-10 (41, 69) or, perhaps, IL-4, IL-13, or TGF-β (1, 2, 38, 70, 71), might possibly have fostered posttreatment relapse in IFN-γ- or CD40L-deficient animals (51, 52, 72). However, outcomes in AmB-treated TNF and IL-12 KO mice are at odds with this consideration, because TNF KO mice are not known to default to a Th2 cell-type response, but relapse after AmB (37), and IL-12p35 KO mice show evidence of default (31), but do not relapse after treatment (Fig. 3). In addition, WT BALB/c mice, induced by immunization to respond to *L. donovani* with an IL-4/IL-10-mediated noncure phenotype (70), also show no late relapse of liver infection after initial AmB treatment (our unpublished observations).

Finally and as suggested above, it is also fair to consider whether the results of this study are drug specific and related only to effects induced by AmB, an agent with recognized immunomodulatory activity (73–76). Sb, unlike AmB, requires T cells, CD40L, IFN-γ, IL-12, and TNF for expression of its in vivo leishmanial effect (31, 34, 37, 40, 45, 46); thus, Sb could not be used to induce near-cure in mice deficient in these factors to set the stage for testing for posttreatment relapse. However, similar to the effects of AmB, there is no late recurrence of *L. donovani* liver infection in Sb-treated ICAM-1 or iNOS KO mice (34, 36). Miltefosine, like AmB, is active initially in T cell- and cytokine-deficient mice infected with *L. donovani* (44); prevention of relapse after miltefosine therapy also requires T cells (77). In preliminary experiments in which IFN-γ and IL-12p35 KO mice were treated during wk 3 with miltefosine (44), liver infection recurred at wk 12 in IFN-γ-deficient, but not IL-12-deficient, mice (not shown), similar to the findings in AmB-treated animals. These results with other forms of antileishmanial chemotherapy suggest that the mechanisms described in this report may be generally applicable to prevention of posttreatment relapse in experimental visceral infection.

**Acknowledgments**

Drs. Shizu Akira and Kiyoshi Takeda (48) originally developed the IL-18−/− mice used in this study.

**Disclosures**

The authors have no financial conflict of interest.

**References**


15. Cortell出现了重复字符，可能导致阅读错误。


