Treatment of Experimental Visceral Leishmaniasis in a T-Cell-Deficient Host: Response to Amphotericin B and Pentamidine

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In experimental visceral leishmaniasis, euthymic but not athymic (nude) BALB/c mice respond to conventional treatment with pentavalent antimony, indicating that the in vivo efficacy of antimony is T cell dependent. This finding correlates with frequent antimony treatment failures for T-cell-deficient patients with visceral leishmaniasis. To determine whether the in vivo efficacies of alternative antileishmanial agents also require T cells, Leishmania donovani-infected euthymic and nude BALB/c mice were treated with pentamidine or amphotericin B. Pentamidine induced leishmanicidal activity in euthymic mice but had little effect in nude mice. In contrast, amphotericin B exerted potent leishmanicidal activities in both euthymic and nude animals. These results suggest that amphotericin B may be of particular use for T-cell-deficient patients with visceral leishmaniasis.

In a prior report (10), we utilized a model of visceral leishmaniasis, athymic (nude) BALB/c mice, and conventional antimony treatment to examine why T-cell-deficient hosts with intracellular infections may fail to respond to appropriate antimicrobial therapy. While pentavalent antimony readily induced leishmanicidal activity in Leishmania donovani-infected euthymic mice, the same treatment produced no effect in nude mice (10). This observation led us to conclude that, although antimony is directly microbicidal against intracellular L. donovani in vitro (9), in vivo responsiveness required host T cells (10). This finding also appeared consistent with the clinical observation that patients with visceral leishmaniasis rendered T cell deficient by iatrogenic immunosuppression or advanced human immunodeficiency virus infection often fail to respond to antimony or promptly relapse after treatment (1, 7, 10).

This background, together with the apparently increasing number of cases of visceral infection in T-cell-deficient patients (1, 7), suggests the need to evaluate alternative treatments for this patient population. Therefore, we have extended our analysis to two other experimentally and clinically active antileishmanial agents, amphotericin B and pentamidine (3, 5, 6, 8, 11–13), and tested their efficacies in L. donovani-infected nude mice.

MATERIALS AND METHODS

Visceral infection. Euthymic (nu/+) and athymic (nu/nu) female BALB/c mice (Life Sciences, Hialeah, Fla.) were infected via the tail vein with 10⁷ L. donovani 1 Sudan amastigotes obtained from infected hamster spleen homogenates (10). The course of visceral infection was assessed microscopically by using stained liver imprints. Liver parasite burdens, expressed in Leishman-Donovan units, were calculated as the number of amastigotes per 1,000 liver cell nuclei × liver weight (in grams) (9, 10).

Treatment. Two weeks after challenge (day 0), liver burdens were determined, and groups of three mice were then treated with pentavalent antimony (9, 10), amphotericin B, or pentamidine administered in 0.2 ml of saline. All mice were sacrificed 1 week later (day +7). Day +7 liver parasite burdens in treated mice were compared with day 0 burdens in controls to determine parasite killing; day +7 burdens in treated mice were compared with day +7 burdens in controls to determine inhibition of parasite replication (9, 10).

As in previous studies (9, 10), 500 mg of antimony (sodium stibogluconate) (Pentostam; Burroughs Wellcome Co., Research Triangle Park, N.C.) per kg of body weight was given once by intraperitoneal injection on day 0 (9, 10). Amphotericin B (Fungizone; E. R. Squibb, Inc., Princeton, N.J.) at 5 mg/kg was injected intraperitoneally on days 0, +3, and +5. Preliminary dose-response experiments with euthymic mice indicated that a single injection of 1 mg of amphotericin B per kg on day 0 had no effect, 1 mg/kg given on days 0, +3, and +5 induced moderate leishmanicidal activity, and 5 mg/kg injected on days 0, +3, and +5 achieved a high level of killing. Pentamidine isethionate (Pentam 300; Lyphomed, Inc., Rosemont, Ill.) at 50 mg/kg was injected intramuscularly on days 0, +3, and +5. For euthymic mice, preliminary experiments with pentamidine showed that (i) three intramuscular injections (days 0, +3, and +5) of 10 or 20 mg/kg induced little or no antileishmanial activity, (ii) three intramuscular injections (same days) of 30 mg/kg produced a moderate leishmanicidal effect, and (iii) the latter effect was modestly enhanced by using 50 mg/kg. Since doses of >50 mg of pentamidine per kg have been reported to be toxic (13), we elected to use three doses of 50 mg/kg in these experiments.

RESULTS AND DISCUSSION

Treatment of euthymic mice with amphotericin B induced effective leishmanicidal activity as judged by the elimination of 89% of liver parasites by day +7 (Table 1). This level of activity was comparable to that achieved by antimony therapy. Pentamidine treatment also induced activity in euthy-
TABLE 1. Treatment effects in euthymic and nude mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver parasite burden (LDU)a</th>
<th>Euthymic mice on day:</th>
<th>Nude mice on day:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>+7</td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>1,102 ± 64</td>
<td>1,695 ± 71 (0)</td>
<td>1,236 ± 48</td>
</tr>
<tr>
<td>Antimony</td>
<td>103 ± 8 (91)</td>
<td></td>
<td>1,529 ± 131 (0)</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>119 ± 15 (89)</td>
<td></td>
<td>325 ± 72 (74)</td>
</tr>
<tr>
<td>Pentamidine</td>
<td>929 ± 32 (16)</td>
<td></td>
<td>1,852 ± 92 (0)</td>
</tr>
</tbody>
</table>

a Beginning 2 weeks after infection (day 0). Antimony, 500 mg/kg on day 0; amphotericin B, 5 mg/kg on days 0, +3, and +5; pentamidine, 50 mg/kg on days 0, +3 and +5.

b The data are means ± standard errors of the means for three experiments with euthymic mice (nine mice per group) and two experiments with nude mice (six mice per group). Percent reduction in liver parasite burden is given in parentheses. LDU, Leishman-Donovan units.

mic animals. While pentamidine achieved only modest killing, visceral parasite replication was completely inhibited.

In contrast to the results for euthymic mice and as previously reported (10), antimony did not induce leishmanicidal effects in nude animals. In addition, pentamidine also failed to induce appreciable leishmanistatic activity in nude mice (Table 1). Amphotericin B therapy, however, was clearly active in nude animals and induced killing with a 74% reduction in liver parasite burdens (day 0 versus day +7).

Therefore, although antimony, pentamidine, and amphotericin B are all active in killing intracellular L. donovani in in vitro models using parasitized macrophages (4, 9), the in vivo antileishmanial efficacies of these three agents differ experimentally with respect to the role of host T cells: optimal responsiveness to antimony and pentamidine requires T cells; responsiveness to amphotericin B does not. The latter finding suggests that amphotericin B, traditionally considered a second-line antileishmanial agent (2, 8, 11, 12), may be worth testing as primary therapy for the T-cell-deficient patient with visceral infection. Lipid-associated amphotericin B preparations (3, 5, 6) may prove even more useful.

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REFERENCES


