Protection of Guinea Pigs against Cutaneous Leishmaniasis by Combined Infection and Chemotherapy

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Received 10 June 1985/Accepted 21 October 1985

A paromomycin and methylbenzethonium chloride ointment cured Leishmania enriettii infections in guinea pigs. Amastigotes were totally eliminated from the treated lesion after 10 days of treatment. A delayed effect also occurred on untreated lesions in the same animals. Lesions treated at various times after infection permitted protective immunity to develop, and 90% of treated animals were refractory to reinfestation.

Immunization against cutaneous leishmaniasis (CL) with living, fully virulent parasites has been used in Iran, Israel, and the USSR (8, 11). This entails inoculating living promastigotes, which leads to normal infection at a selected site on the body and at a suitable time. Microbial contamination, allergic reactions, and the production of chronic disease of many years' duration can occur in addition to lesion formation (6, 19). The development of effective topical treatment for CL (4), together with the finding that acquired protection develops before the complete healing of lesions (2, 12, 14), suggested the combined use of infection and chemotherapy in treating protection.

Leishmania enriettii in guinea pigs is used as a laboratory model of human CL (2, 17, 18, 20), since lesions usually self-cure and cause protection. A cell-mediated immune response (2, 10) and production of antibody (3, 15) to leishmanial antigen has been demonstrated in infected and convalescent animals. Like CL in humans, the disease in guinea pigs can exhibit a clinical spectrum ranging from simple, self-curing lesions to chronic metastatic disease (2, 5, 13).

This study in guinea pigs was aimed at determining the effect of topical treatment, given at various times after infection, on the development of protection against CL.

L. enriettii LRC-L327 promastigotes were used to infect and challenge guinea pigs. One or both ears were injected intradermally with 2 × 10^6 promastigotes. Lesion development was followed by making stained smears and subcultures at different times after infection, as previously described (4). Lesion size was measured (in millimeters) in two diameters (D and d) at right angles to each other, and the lesion size (S; in square millimeters) was determined according to the formula: S = (D × d)/2.

PR ointment containing 15% paromomycin and 12% methylbenzethonium chloride in soft white Paraffin (United Kingdom patent no. GB 2117237 A; analyzed and supplied by Teva Pharmaceutical Industries, Jerusalem) was highly effective against L. enriettii in guinea pigs. After 10 days of treatment, the parasites were eliminated from the treated ears, and the lesions had healed (Fig. 1). No relapse of the disease was demonstrated in the treated guinea pigs after termination of treatment. In the control group, the parasites generally disappeared spontaneously from the site of infection within 70 to 100 days after infection. When both ears of the guinea pigs were infected and only the right ear was treated, a delayed effect was also observed on the untreated lesion of the left ear. These had cured by 30 days after the end of treatment of the right ear (Fig. 2). However, when a susceptible guinea pig with well-developed lesions and metastases was treated, most of the parasites were eliminated from the treated ear after 10 days of treatment. Twenty days were required to eliminate all of the parasites, and no effect was seen on the parasites in the lesion of the untreated left ear or in the metastatic lesions.

Table 1 shows the production of antileishmanial antibodies in infected treated and untreated guinea pigs, as determined by a radioimmunoassay with the supernatant fraction of freeze-thawed and sonicated L. enriettii promastigotes as the coating antigen. Antibody titers did not rise significantly in the infected untreated animals during the first 4 weeks, being 3.5 times higher than that in uninfected untreated controls. This level rose to 6.4 times by 6 weeks and 10.2 times by 8 weeks. Treatment given from 4 weeks onward neither abolished nor reduced antibody production in the treated guinea pigs. Sera from infected treated animals, tested 13 weeks after infection, showed 1.33 to 1.88 times more antibody than did sera from infected, spontaneously cured guinea pigs.

To measure the development of delayed-type hypersensitivity, two types of leishmanin were tested: one made with L. enriettii promastigotes and the other made with Leishmania donovani promastigotes made up in 0.5% phenol–saline at 10^6 promastigotes per ml. Twenty-four hours after intradermal inoculation of leishmanin, slight induration was seen.
in both infected treated and untreated animals to both *L. enrietti* and *L. donovani* antigen (Table 1). This response increased slightly in intensity after another 24 h, showing highest activity in guinea pigs treated 4 weeks after infection. The response to *L. donovani* antigen was slightly higher. There was no response to the phenol-saline control in any of the guinea pigs.

All of the guinea pigs, including the self-curing, untreated animals, were found to be resistant to reinfection with 5 × 10⁶ recently isolated *L. enrietti* promastigotes injected into the left ear 17 weeks after infection, except for the two that received treatment during the first 4 weeks of infection. No lesions developed, and parasites were not detected. The uninfected normal control animals all became infected after challenge, developing persistent lesions.

Treated guinea pigs were cured locally of parasites after 10 days of treatment with PR ointment. That a delayed effect was seen on the untreated lesions only in guinea pigs with simple CL, but not in a guinea pig with metastases, suggests that the delayed effect resulted from the development of an immune response rather than through penetration of the drug. A similar phenomenon has also been observed in experimentally infected, treated BALB/c mice (4) and in human CL patients treated locally by cryotherapy (1).

Antileishmanial vaccine consisting of live virulent promastigotes is the only vaccine presently available. This poses problems, since the process mimics long-term natural infections (6). The development of an easily applied topical treatment with minimal side effects (4) and the effect described here in guinea pigs suggests that long-term infection

<table>
<thead>
<tr>
<th>TABLE 1. Antileishmanial response of guinea pigs to infection with <em>L. enrietti</em>[^a]</th>
<th></th>
<th>Antibody titers (±SD) (experimental/control)</th>
<th>Skin test score[^b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (wk after infection)</td>
<td>Before</td>
<td>After</td>
<td>13wk after</td>
</tr>
<tr>
<td></td>
<td>treatment</td>
<td>treatment</td>
<td>infection</td>
</tr>
<tr>
<td>2</td>
<td>4.64 ± 1.6</td>
<td>7.84 ± 2.2</td>
<td>4.29 ± 1.3</td>
</tr>
<tr>
<td>4</td>
<td>4.94 ± 0.5</td>
<td>9.13 ± 3.9</td>
<td>11.65 ± 3.3</td>
</tr>
<tr>
<td>6</td>
<td>8.64 ± 4.0</td>
<td>13.72 ± 2.5</td>
<td>17.31 ± 3.7</td>
</tr>
<tr>
<td>8</td>
<td>13.78 ± 6.4</td>
<td>14.67 ± 5.2</td>
<td>8.28 ± 3.4</td>
</tr>
<tr>
<td>10</td>
<td>10.40 ± 1.6</td>
<td>7.77 ± 1.2</td>
<td>10.14 ± 1.8</td>
</tr>
<tr>
<td>Infected, untreated</td>
<td>8.56 ± 1.7[^c]</td>
<td>6.18 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>Uninfected, treated</td>
<td>1.17 ± 0.5</td>
<td>1.12 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Uninfected, untreated</td>
<td>1.53 ± 0.3</td>
<td>2.98 ± 1.34</td>
<td></td>
</tr>
</tbody>
</table>

[^a]: Animals were treated topically at various times after being infected. The infected ear of each animal was treated for 10 days. Each group comprised 4 animals.

[^b]: ± ± + + correspond to no induration and palpable, definite, and very obvious induration, respectively. Numbers indicate numbers of guinea pigs. Tests were done 16 weeks after infection.

[^c]: Titer was determined 55 days after infection.
during vaccination could probably be shortened from months to weeks.

The most accurate way of measuring the development of resistance is to check the ability of hosts to resist challenge infections. Our results indicated that treatment applied 6 weeks, and in most cases even as early as 2 weeks, after infection was not detrimental to the acquisition of protective immunity. The delayed hypersensitivity developed by guinea pigs in this study, although not very strong, developed as early as 4 weeks after infection. Interestingly, the response to L. donovani antigen was slightly higher than that with the homologous L. enriettii antigen.

Although antileishmanial antibodies in humans (20) or guinea pigs (13, 15, 16) do not appear to play a significant role in the development of resistance, they are said to have an important function in healing the primary lesion (5) and can therefore be used to indicate the development of an immune response. The results presented in this study revealed that treatment given at different times starting 4 weeks after infection neither stopped nor abolished antibody production, and their levels remained as high as that in the spontaneously cured, untreated animals at 13 weeks after infection.

The loss of resistance to reinfection after treatment has only been demonstrated in a few cases. Guirges (7) has reported reinfection in 8 out of 3,420 Iraqi patients that had recovered from Leishmania tropica infections. Seven of these eight patients were either under steroid treatment or had been treated chemotherapeutically before their initial lesions healed. In addition, Marzinowsky and Schourenkoff (9) reported experimental reinfection after the surgical removal of active lesions. These examples are too small in number to draw definite conclusions concerning the correlation between treatment and loss of resistance to reinfection. Also, none of the 200 or more patients treated during the last several years at the Hadassah Hospital, including the many living in endemic areas (J. El-On, unpublished data) has become reinfected. This observation, together with the findings presented in this study, suggests that combined infection followed by chemotherapy should be considered in antileishmanial vaccination. Further study must be done in humans to discern the correlation between duration of infection, treatment, and development of protective immunity.

We thank Graciela Rozen for her technical assistance.

This work was supported by the leishmaniasis component of the United Nations Development Program/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases.

LITERATURE CITED


