The Indian langur: preliminary report of a new nonhuman primate host for visceral leishmaniasis*

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Described are the susceptibility of the Indian langur (Presbytis entellus) to Leishmania donovani and the consequent haematological and serum biochemical changes. The host response to antileishmanial chemotherapy and the immunological profile were also examined. Each langur was inoculated intravenously with 1 x 10⁶ amastigotes; a spleen biopsy carried out on day 35 post-infection (p.i.) revealed 10–13 L. donovani bodies per 500 cell nuclei, which reached a maximum of 130–195 at death (day 105–110 p.i.). The infected monkeys lost body weight, developed severe anaemia, lymphocytosis, hyperproteinaemia, hypergammaglobulinaemia, hypoalbuminaemia and an increase in the level of alkaline phosphatase and alanine aminotransferase (AAT).

Treatment with sodium stibogluconate (60 mg Sb⁵⁺ per kg body weight intramuscularly for 10 days) reduced the number of spleen parasites (0–1 amastigotes per 500 cell nuclei) but after the therapy the parasites appeared in the skin, which had previously been free of infection. Relapse occurred on day 30 post-treatment (10–24 amastigotes per 500 cell nuclei) and the parasites were resistant to repeat intensive therapy (120 mg Sb⁵⁺ per kg per day x 30 days). The stibogluconate treatment caused a proportionate reduction in the haematological and biochemical parameters to normal values except for alkaline phosphatase and AAT, which remained elevated. The level of IgG antibodies, which rose during the infection, rapidly fell to the pretreatment value following the first therapeutic schedule and then increased a second time coinciding with relapse.

Our findings suggest that langurs could serve as acceptable models for human visceral leishmaniasis.

Introduction

Hamsters and mice are frequently used for the primary screening of potential antileishmanial compounds; however, the results obtained with these animals are difficult to extrapolate to humans. Compound(s) that are active in rodents therefore need to be screened also in higher animals, such as dogs, cats and non-human primates. Monkeys, which are phylogenetically and physiologically close to humans seem to offer advantages in this respect (1, 2). Earlier studies carried out on the susceptibility of nonhuman primates to Leishmania donovani were somewhat limited (3–5). More recently the marmoset (Callithrix jacchus) (6), the owl monkey (Aotus trivirgatus) (7), and the squirrel monkey (Saimiri sciureus) (2, 8–10) have been suggested as potentially useful nonhuman primates for the investigation of visceral leishmaniasis. The owl monkey develops fulminating infections with the Khartoum strain of L. donovani (7), while the squirrel monkey develops less acute infections; in both these species, the infection is fatal by day 28 and day 50 post-infection (p.i.), respectively. Several Old World monkeys such as Macaca mulatta, M. fasciolaris and M. nemestrina have also been investigated, but resulted in low and inconsistent infections (9).

The present article describes the results of a preliminary investigation of the langur (Presbytis entellus) as an experimental model for visceral leishmaniasis. Attempts were directed at establishing
L. donovani infection in these monkeys (11), which, as well as being closely related to humans, are readily available in India. To establish the relevance of the model for human visceral leishmaniasis, we required basic information on the host–parasite relationship. We therefore gathered data on the haematology, blood chemistry, immunology, chemotherapy, and histopathology of langurs during the course of L. donovani infection.

Materials and methods

Animals and inoculation
Young, male wild-caught langur monkeys (3–4 kg) were bought from an animal contractor. These were caged individually and housed in an environmentally controlled primate house. The langurs were quarantined for 45 days before the experiments began, and were fed on commercially available primate feed supplemented with seasonal fruits and vegetables. Water was provided ad libitum.

L. donovani amastigotes (Dd8 strain) were isolated from the spleens of heavily-infected hamsters (40–60-day-old infection); the inoculum was prepared as described previously (12). Seven langur monkeys were each inoculated intravenously in the forearm with 1 x 10^8 amastigotes. The general condition of the animals was kept under constant surveillance during the entire study period.

Susceptibility studies

Assessment of parasites. In vivo: Splenic tissue from five monkeys, obtained at biopsy on day 35 p.i. (12), was dab-smeared, stained with Giemsa, and examined for amastigotes. Subsequent assessment of parasites was carried out similarly on days 49, 64, and 80 p.i.

Impression smears of a skin snip cut from the site of incision were made and assessed for parasites. The incisions were sutured with catgut and adequate amounts of antibiotics (neomycin–bacitracin sulfacetamide powder and nitrofurazone ointment) applied locally. In addition, the animals were intraperitoneally injected with oxytetracycline (50 mg per animal) once per day for 5 days.

Since incision of an infected organ promotes multiplication of leishmanial parasites (13), for the remaining two monkeys, which received no surgical intervention, the parasitic load and longevity were assessed by autopsy.

In vitro: The presence of parasites in the spleen and skin of the monkeys was also confirmed by in vitro culture. Cultures were performed in NNN-medium vials, with RPMI-1640 as an overlay (12). After 7–10 days of incubation at 25 °C the vials were checked for growth of promastigotes.

Chemotherapeutic studies

After the initial parasite assessment (on day 35 p.i.), two langurs that had 11–12 parasites per 500 cell nuclei were treated intramuscularly with sodium stibogluconate at a dose schedule of 60 mg Sb5+/kg per day for 10 days (day 40 to day 50 p.i.). The remaining three monkeys served as untreated controls. The first post-treatment spleen biopsy to evaluate the parasite burden by the dab-smear method was carried out 10 days after the last therapeutic dose; one untreated monkey died at this stage. Twenty days later (on day 80 p.i.) the two treated and two untreated monkeys were re-examined for parasites to verify whether relapse had occurred. Since relapse occurred in both the treated monkeys, a second therapeutically schedule at a higher dose (120 mg Sb5+/kg) was initiated on day 90 p.i. and continued till day 120 p.i. (30 days).

Because one animal from the treated group and the untreated control group died after completion of the second therapeutic regimen (on day 120 p.i.), the survivors in both groups (one in each) were autopsied on the same day. Dab-smears of the liver, spleen, bone marrow, skin, kidney, intestine, blood and lung were also made to determine the parasite distribution under drug pressure.

The efficacy of sodium stibogluconate was expressed as the percentage inhibition of parasite multiplication (12) and in terms of LDU (L. donovani units), as described by Hanson et al. (14).

Haematology

The changes in haematology during the infection and after chemotherapy were studied at different times. Blood collected from the femoral vein in ethylene-diaminetetraacetic acid was used to determine the mean corpuscular volume (MCV), total erythrocyte (RBC) and leukocyte (TLC) counts, haematocrit (HCT), and haemoglobin (Hb) level in a semiautomatic counter (model HC-555, Boehringer Mannheim Diagnostics); platelets were assayed in a Neubauer counting chamber; and differential leukocyte counts were performed on blood smears that had been stained with Leishman’s stain.

Blood chemistry

The following levels were determined at various intervals: blood urea nitrogen (15), blood glucose (16), alanine aminotransferase (AAT) (17), total protein (18), albumin (19), alkaline phosphatase (20), serum cholesterol (21), serum bilirubin (22), and serum creatinine (23).
Immunology

Leishmanin test (DTH). The source of antigen was L. donovani promastigotes maintained in vitro in NNN culture-tubes with RPMI-1640 medium as an overlay. The antigen was prepared by suspending the promastigotes in 0.5% formalin–saline. Infected and treated monkeys were each injected intradermally on the left forearm with 0.1 ml of leishmanial antigen (1 x 10⁷ per ml). The skin reaction (induration) was observed for three consecutive days: a wheal of more than 5-mm diameter was considered positive (10).

Humoral response (gel diffusion). The total concentrations of serum IgM and IgG were determined using the single radial immunodiffusion technique (24), with commercially available plates and antisera to human IgM and IgG. For IgM, neat sera were used and for IgG, 1:10 reference sera. The incubation time for IgM was 72 hours and for IgG, 50 hours (10).

Pathology

Gross and microscopic examinations of the major organs were performed at autopsy following the death of the animals.

For histology, the tissues were fixed in 10% formalin–saline, embedded in paraffin, cut into sections of 5 μm thickness, and stained with haematoxylin and eosin. Sections of skin were stained additionally with Giemsa.

Results

Clinical observations

All the infected langurs became listless and weak about 2 months after infection; progressive deterioration in their condition continued till death. Loss of body weight (about 1 kg) occurred during the course of the infection. In infected untreated langurs, decoloration of the facial skin, which was more prominent under the eyes, was observed around day 90 p.i. (Fig. 1). The face was also puffy; no such change occurred in infected treated monkeys.

Susceptibility studies

All the animals developed infection. By day 35 p.i. the amastigotes could be quantitated in spleen imprints. The splenic parasites in five langurs were, on average, 11.4 ± 1.5 per 500 cell nuclei (Table 1, Fig. 2), and reached a maximum (178 ± 24 per 500 cell nuclei) at death (day 110 p.i.). The two langurs

on which no spleen biopsy was performed died on day 106 ± 2; the parasite burden in their spleens was 137.5 ± 10.6.

The proliferation of promastigotes in culture-tubes seeded with infected tissue on day 7 after inoculation confirmed the in vivo results.

Chemotherapeutic studies

Use of sodium stibogluconate in the first therapeutic schedule resulted in 99.1 ± 1.2% inhibition of parasite multiplication on day 10 after administration. Repeat spleen biopsy on day 30 post-treatment revealed a slight increase of parasites (% inhibition, 92.3 ± 3.6). The second therapeutic schedule failed to reduce the parasitic load (% inhibition, 88.25 ± 9.6) (Table 1). Following the therapy, the parasites appeared in the skin, which had previously been free of infection (Table 2).

Of the three infected and untreated monkeys, one died during biopsy on day 60 p.i. (with 46 amastigotes per 500 cell nuclei). The second monkey died on day 110 p.i. and the third, sole survivor was autopsied on day 120 p.i.; the parasite load in the spleen of these two monkeys was 161–195 per 500 cell nuclei. The skin was free of parasites.

a Tripartigen, Hoechst AG, Frankfurt am Main, Germany.
**Fig. 2.** Giemsa-stained dab-smear (x 100) of infected langur spleen (day 35 post-infection), showing *Leishmania donovani* amastigotes (arrows).

**Distribution pattern of parasites following therapy**

At the end of the study the spleen, liver and bone marrow of the treated animals had a reduced number of parasites; the kidney, intestine, blood and lungs were almost free of parasites (Table 2). However, samples of skin from the dorsum and eyelids of these animals had considerably more parasites compared with the untreated controls.

**Haematology**

The haematological results are shown in Table 3. The most consistent feature of the infected langurs was microcytic anaemia; the appreciable fall in red blood cell count and haemoglobin level occurred from day 49 p.i. onward. The haematocrit also decreased during the course of the infection, and was minimum at the end of the study (day 110 p.i.). The leukocyte count, although reduced, exhibited a wide variation. One animal showed a reversal of the polymorphonuclear ratio during the last phase of infection (day 90 p.i. onward). The remaining haemogram values were within the normal range.

Sodium stibogluconate therapy supported gradual recovery in the red blood cell count, haemoglobin levels and haematocrit, while the leukocyte count reached the pre-infection level.

**Table 1: Susceptibility of the langurs to Leishmania donovani and their response to chemotherapy**

<table>
<thead>
<tr>
<th>No. of days post-infection:</th>
<th>35 (biopsy 1)</th>
<th>60 (biopsy 2)</th>
<th>80 (biopsy 3)</th>
<th>110–120 (autopsy)</th>
<th>Survival time (days post-infection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>Parasite burden b</td>
<td>Body weight (kg)</td>
<td>Parasite burden b</td>
<td>Body weight (kg)</td>
<td>Parasite burden b</td>
</tr>
<tr>
<td><strong>Treated group</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td>12.0</td>
<td>4.2</td>
<td>1.0 (98.2)c</td>
<td>5.1</td>
<td>20.0 (89.7)</td>
</tr>
<tr>
<td>4.4</td>
<td>12.0</td>
<td>4.5</td>
<td>0 (100)</td>
<td>5.2</td>
<td>10.0 (94.9)</td>
</tr>
<tr>
<td><strong>Untreated controls</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4.7</td>
<td>11.0</td>
<td>4.6</td>
<td>46.0</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>4.1</td>
<td>13.0</td>
<td>4.0</td>
<td>54.0</td>
<td>3.9</td>
<td>190.0</td>
</tr>
<tr>
<td>4.6</td>
<td>9.0</td>
<td>4.5</td>
<td>45.0</td>
<td>3.8</td>
<td>160.0</td>
</tr>
<tr>
<td>3.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
</tbody>
</table>

*a* First schedule: sodium stibogluconate (16 mg Sb\(5^+\) per kg per day for 10 days) from day 40 to day 50 post-infection; second schedule: sodium stibogluconate (120 mg Sb\(5^+\) per kg per day for 30 days) from day 90 to day 120 post-infection.

*b* No. of amastigotes per 500 cell nuclei of spleen macrophages.

*c* Figures in parentheses are the % inhibition of parasite multiplication in the spleens of treated monkeys.
Table 2: Distribution of *Leishmania donovani* in the langurs on autopsy

<table>
<thead>
<tr>
<th>Day of observation</th>
<th>No. of amastigotes per 500 cell nuclei in:</th>
<th>No. of amastigotes per 100 fields in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spleen</td>
<td>Liver</td>
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<tr>
<td>Treated group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>120</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>(0.13 x 10⁹)</td>
<td>(1.4 x 10⁹)</td>
</tr>
<tr>
<td>2</td>
<td>120</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>(0.03 x 10⁹)</td>
<td>(0.2 x 10⁹)</td>
</tr>
<tr>
<td>Untreated controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>120</td>
<td>161</td>
</tr>
<tr>
<td></td>
<td>(2.6 x 10⁹)</td>
<td>(1.6 x 10⁹)</td>
</tr>
<tr>
<td>2</td>
<td>110</td>
<td>195</td>
</tr>
<tr>
<td></td>
<td>(3.9 x 10⁹)</td>
<td>(3.7 x 10⁹)</td>
</tr>
<tr>
<td>3</td>
<td>105</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>(2.3 x 10⁹)</td>
<td>(2.5 x 10⁹)</td>
</tr>
<tr>
<td>4</td>
<td>108</td>
<td>145</td>
</tr>
<tr>
<td></td>
<td>(2.5 x 10⁹)</td>
<td>(3.1 x 10⁹)</td>
</tr>
</tbody>
</table>

* Figures in parentheses are the values expressed as LDU (see ref. 14).

Table 3: Haematological changes in the langurs during the course of *Leishmania donovani* infection and after therapy

<table>
<thead>
<tr>
<th>No. of days post-infection</th>
<th>RBC (per mm³)</th>
<th>Hct (%)</th>
<th>Hb (g/dl)</th>
<th>MCV (x10⁻¹⁵ l)</th>
<th>TLC (cells per mm³)</th>
<th>Leukocyte count (%)</th>
<th>Platelet count (per mm³)</th>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>B</td>
<td>L</td>
</tr>
<tr>
<td>Untreated controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5.77±0.31 (5)²</td>
<td>36.98</td>
<td>12.08</td>
<td>94.0</td>
<td>8420</td>
<td>55.2</td>
<td>42.8</td>
</tr>
<tr>
<td>49</td>
<td>4.75±0.7 (3)</td>
<td>38.82</td>
<td>12.00</td>
<td>82.6</td>
<td>8240</td>
<td>41.6</td>
<td>56.3</td>
</tr>
<tr>
<td>64</td>
<td>4.86±0.07 (2)</td>
<td>40.55</td>
<td>9.9</td>
<td>83.0</td>
<td>8350</td>
<td>47.0</td>
<td>57.0</td>
</tr>
<tr>
<td>90</td>
<td>3.17±0.23 (2)</td>
<td>33.30</td>
<td>8.75</td>
<td>95.0</td>
<td>10 950</td>
<td>50.0</td>
<td>48.0</td>
</tr>
<tr>
<td>110</td>
<td>2.71±0.71 (1)</td>
<td>15.6</td>
<td>5.4</td>
<td>72.0</td>
<td>5200</td>
<td>25.0</td>
<td>74.0</td>
</tr>
<tr>
<td>Treated group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>4.07±0.02 (2)</td>
<td>32.5</td>
<td>8.25</td>
<td>79.5</td>
<td>9800</td>
<td>42.0</td>
<td>52.5</td>
</tr>
<tr>
<td>90</td>
<td>4.82±0.40 (2)</td>
<td>42.00</td>
<td>13.70</td>
<td>90.0</td>
<td>9650</td>
<td>57.0</td>
<td>40.0</td>
</tr>
</tbody>
</table>

* RBC = total erythrocyte count; Hct = haematocrit; Hb = haemoglobin; MCV = erythrocyte volume; TLC = total leukocyte count.
* B = basophils; L = lymphocytes; E = eosinophils; M = monocytes.
* Figures in parentheses are the number of langurs.
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**Blood chemistry**
The infected animals exhibited a significant reduction in total protein and albumin levels but an increase in globulin from day 64 p.i. onward. The levels of AAT and alkaline phosphatase were also elevated by day 35 p.i., but the cholesterol level declined. These results indicate liver dysfunction. Following therapy with sodium stibogluconate both the total protein level and the albumin:globulin ratio were restored to pre-infection levels (Table 4).

**Immunology**
In infected langurs there was a significant increase in the IgG level by day 50 p.i. (246 mg/ml), which peaked at >310 mg/ml upon death (Fig. 3). In treated langurs a steep rise in the IgG level occurred on day 70 p.i. (after first post-treatment biopsy).

During the course of active infection and following therapy, the monkeys gave inconclusive skin tests with *Leishmania* spp.

**Pathology**

**Gross.** Splenomegaly and hepatomegaly occurred in all the infected monkeys. In two monkeys the mean weight of the spleen increased 7-fold and that of the liver 1.5-fold (Fig. 4). Sodium stibogluconate therapy reduced the size of the spleens, but the size of the livers remained unaffected. Langurs of the same age, weight, and sex that were sacrificed for other experiments exhibited normal livers and spleens.

**Microscopy.** Liver. *L. donovani* bodies were observed in Kupffer cells. There was fatty degeneration of parenchymatous cells, and the portal areas were infiltrated with lymphocytes and macrophages. Spleen. There was marked congestion, and reticular cells were proliferated with cytoplasmic *L. donovani* bodies. The pulp contained an increased number of plasma cells and there was hyperplasia of the follicles. Skin. The skin of infected monkeys appeared normal. In the infected treated animals, sections of skin exhibited collections of *L. donovani* bodies in the subpapillary zone of the dermis, with a minimum increase of histiocytes.

**Discussion**
The major disadvantage associated with rodent models is that their metabolism and drug kinetics

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**Table 4: Blood biochemical changes in the langurs during the course of *Leishmania donovani* infection and after therapy**

<table>
<thead>
<tr>
<th>No. of days post-infection</th>
<th>Total cholesterol (mg/dl)</th>
<th>Alkaline phosphatase (IU/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>AAT* (IU/dl)</th>
<th>Bilirubins (mg/dl)</th>
<th>Blood urea (mg/dl)</th>
<th>Blood glucose (mg/dl)</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
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<tbody>
<tr>
<td><strong>Untreated controls</strong></td>
<td></td>
<td></td>
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<tr>
<td>0</td>
<td>167.40 ±14.61 (5)b</td>
<td>5.14 ±1.32</td>
<td>0.96</td>
<td>19.40 ±5.98</td>
<td>0.52 ±0.14</td>
<td>31.40 ±8.47</td>
<td>97.2 ±17.23</td>
<td>6.90 ±0.32</td>
<td>4.76 ±0.64</td>
<td>3.04 ±0.61</td>
</tr>
<tr>
<td>49</td>
<td>99.20 ±6.98 (3)</td>
<td>7.70 ±2.28</td>
<td>1.6</td>
<td>92.00 ±3.41</td>
<td>1.6 ±0.1</td>
<td>26.8 ±6.3</td>
<td>107.0 ±28.6</td>
<td>8.56 ±0.63</td>
<td>2.68 ±0.33</td>
<td>5.62 ±0.60</td>
</tr>
<tr>
<td>64</td>
<td>133.00 ±8.49 (2)</td>
<td>16.00 ±2.83</td>
<td>1.45</td>
<td>68.50 ±4.78</td>
<td>0.95 ±0.9</td>
<td>31.5 ±12.0</td>
<td>85.5 ±7.8</td>
<td>5.30 ±0.57</td>
<td>1.70 ±0.14</td>
<td>3.70 ±0.57</td>
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<tr>
<td>90</td>
<td>66.0 (1) ±6.00</td>
<td>13.00 ±1.8</td>
<td>1.8</td>
<td>65.00 ±1.5</td>
<td>0.6 ±0.1</td>
<td>40.0 N.A.c</td>
<td>6.0 ±3.25</td>
<td>2.5 ±0.57</td>
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<td></td>
</tr>
<tr>
<td>110</td>
<td>90.0 (1) ±6.30</td>
<td>27.00 ±1.8</td>
<td>1.8</td>
<td>60.00 ±1.5</td>
<td>1.5 ±0.1</td>
<td>N.A. ±3.8</td>
<td>N.A. ±1.5</td>
<td>2.3 ±0.57</td>
<td></td>
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<td><strong>Treated groups</strong></td>
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<td></td>
<td></td>
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<tr>
<td>64</td>
<td>107.50 ±8.19 (2)</td>
<td>5.25 ±2.47</td>
<td>1.1</td>
<td>45.00 ±0.97</td>
<td>0.65 ±0.07</td>
<td>26.0 ±6.5</td>
<td>99.5 ±34.6</td>
<td>5.15 ±0.21</td>
<td>1.55 ±0.21</td>
<td>3.60 ±0.41</td>
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<tr>
<td>90</td>
<td>72.50 ±6.30</td>
<td>16.00 ±1.25</td>
<td>1.25</td>
<td>45.00 ±7.09</td>
<td>0.9 ±0.1</td>
<td>43.5 N.A.</td>
<td>6.05 ±3.55</td>
<td>2.50 ±0.64</td>
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* AAT = alanine aminotransferase.

b Figures in parentheses are the number of langurs.

* N.A. = not available.
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Fig. 3. Changes in the concentration of serum IgG in langurs during *Leishmania donovani* infection and following therapy with sodium stibogluconate (values are the mean for two monkeys in each group).

Fig. 4. Illustration of the changes in weight of liver and spleen of langurs during *Leishmania donovani* infection and following therapy with sodium stibogluconate (values are the mean for two monkeys).

differ markedly from those of humans. The results obtained from primary screening of drugs must therefore be confirmed by secondary or tertiary screening in higher animals. Use of primates for this purpose would offer enormous advantages.

Efforts have been made to establish leishmaniasis in dogs (25) and wild-caught opossums (*Didelphis marsupialis*) (26). Among primates the New World monkeys *A. strivirgatus* (owl monkeys) (7) and *S. sciureus* (8) are susceptible to the *L. donovani* complex. Owl monkeys are susceptible to the Khartoum strain of *L. donovani*, developing fulminating leishmaniasis by day 18–24 p.i. The infection is highly pathogenic, and the animals develop morbidity even before patency occurs. Chapman et al. have, however, established the usefulness of this model for chemotherapy studies (27). *S. sciureus* is susceptible to visceral leishmaniasis (8), although the infection is less acute, and morbidity occurred relatively late (41–52 days p.i.). Madindou et al. have established the therapeutic efficacy of antimony compounds using *S. sciureus* (9).

Studies on Old World monkeys have been very scant, and a working model has not yet been developed (9). Recently we have observed that the “intake” of *L. donovani* by rhesus monkeys varies, even after triple inoculations (1x10⁶ amastigotes given at 1-week intervals), and a single inoculum was to no avail (J.C. Katiyar, unpublished results, 1991). Langurs are phylogenetically and physiologically similar to humans and in preliminary investigations have been found to be susceptible to *L. donovani* infection (11). This could make them a potential model for visceral leishmaniasis.

In human leishmaniasis the clinical picture characteristically consists of anaemia, progressive emaciation, intermittent fever, hepatomegaly, splenomegaly, lymphadenopathy and hypergammaglobulinaemia. The infected langurs in the present study developed anaemia and progressive loss of
body weight. Upon autopsy, hepatomegaly and splenomegaly were found; these symptoms have also been observed in squirrel and owl monkeys. However, the depigmentation of facial skin, particularly of the area around the eyes, was a feature restricted to langurs only.

In humans, the course of L. donovani infection is usually progressive until the patient dies of secondary infections or haemorrhage (28), but ranges from a mild self-curing to a severe fatal disease (29). The langurs appeared to simulate the course in humans and developed mild-to-severe infection, surviving for 104–110 days, followed by death. Compared with owl and squirrel monkeys, langurs survive sufficiently long to permit ample time for study.

Clinically, sodium stibogluconate is still the drug of choice; the recommended dose to treat Indian patients with kala-azar is 20 mg Sb5+/kg given intramuscularly for at least 40 days (30). In langurs the requisite amount was calculated using a conversion factor of three (31), and a 10-day regimen of 60 mg Sb5+/kg initiated on day 40 p.i. against established infection almost cured the monkeys, eliciting a response very similar to that in humans. However, the infection relapsed after a month. Repeat therapy using a higher dose did not appear to bring about any improvement, although this observation is based on the results from only a few animals. Following sodium stibogluconate therapy, L. donovani parasites were found in large numbers in the skin of the langurs, a situation that develops in cases of human post-kala-azar dermal leishmaniasis (32).

The haematological changes that occur during the course of visceral leishmaniasis in humans (33), owl monkeys and squirrel monkeys (2) are similar, and are characterized by microcytic anaemia, leukopenia and, in some cases, lymphocytosis/lymphomonocytosis. A similar peripheral blood picture was observed also in langurs.

Langurs also developed hypergammaglobulinaemia, hypoalbuminaemia, hyperprotninaemia, with reversal of the IgA:IgG ratio on day 35 of the infection. These changes parallel those of visceral leishmaniasis in humans. However, during the terminal phase of infection, the langurs exhibited hypoproteinaemia, which could have been due to exhaustion and their moribund condition. A significant increase was also observed in the levels of AAT and alkaline phosphatase, which became more marked before death, denoting liver disorders. Increasing evidence suggests that cell-mediated immunity plays a major role in human visceral leishmaniasis, while the humoral component has only a minor function (34–36). Skin-test responses to leishmanin occur only after therapy and may take from a few weeks to 2 years to become positive (10, 37–40). This could explain the absence of skin reactions in the monkeys.

In humans a significant increase in serum gammaglobulins (primarily IgG) during the course of leishmaniasis has been reported. An increase in the level of serum IgG was also observed in the langurs, but this returned to the pretreatment level after therapy, and upon relapse. The postmortem findings indicate the similarity of the infection in langurs and humans and suggest their usefulness as a secondary/tertiary model for visceral leishmaniasis.

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Résumé

L’entelle (Presbytis entellus): rapport préliminaire sur un primate non humain comme modèle pour la leishmaniose viscérale

Le kala-azar est une maladie souvent mortelle si elle est négligée. Il n’existe aucune mesure de lutte contre cette maladie, et les quelques agents thérapeutiques dont on dispose sont peu efficaces et potentiellement toxiques. La recherche d’agents prophylactiques ou curatifs se heurte à l’absence de systèmes de criblage appropriés. Bien que les rongeurs puissent convenir pour le criblage primaire, les résultats obtenus ne sont pas facilement extrapolables à l’homme. Il est donc nécessaire de disposer d’un système fiable de criblage secondaire et tertiaire afin de confirmer les résultats obtenus sur les modèles primaires. Les singes, phylogénétiquement et physiologiquement plus proches de l’homme, pourraient servir de modèle pour la leishmaniose.

Nous rapportons ici les résultats d’une étude préliminaire de la sensibilité de Presbytis entellus (entelle ou langur) à l’infection par Leishmania donovani. Pour étudier l’acceptabilité de ce...
modèle, nous avons rassemblé des données sur l'hématologie, la chimie sanguine, l'immunologie, la chimiothérapie et l'histopathologie des entelles infectées par *L. donovani*.

Après inoculation intraveineuse de 1 x 10^8 amastigotes aux singes, une biopsie de la rate réalisée le 35e jour suivant l'inoculation a montré la présence de 10–13 leishmanies (*L. donovani*) par 500 noyaux cellulaire, et jusqu'à un maximum de 130–195 leishmanies par 500 noyaux au moment de la mort des animaux 105 à 110 jours après l'inoculation. Les singes infectés présentaient une perte de poids, une dépigmentation de la peau de la face, avec oedème périoculaire, une anémie sévère, une lymphocytose, une hyperprotéïnémie, une hypergammaglobulinémie, une hypoaortinémie et une augmentation des taux de phosphatase alcaline et d'alanine-amino transférase révélant une atteinte hépatique. Un traitement intramusculaire par le stibogluconate de sodium (20 mg Sb5+ par kg de poids corporel pendant 10 jours) réduisait la charge parasitaire dans la rate (0–1 amastigote par 500 noyaux cellulaires). Toutefois, après le traitement, les parasites réappaissaient dans la peau, qui avait été jusqu'alors indemne d'infection. La rechute survenait 30 jours après le traitement (10–24 amastigotes par 500 noyaux de cellules spléniques) et était rebelle au traitement, même répété et intensif (40mg Sb5+ par kg de poids corporel par jour pendant 30 jours). Le traitement par le stibogluconate de sodium entraînait un retour des paramètres hématologiques et biochimiques à la normale, sauf pour la phosphatase alcaline et l'alanine-amino transférase, qui restaient élevées. Les IgG, qui avaient augmenté au cours de l'infection et lors de la première étape du traitement, retombaient rapidement aux niveaux précédant le traitement et remontaient lors de la rechute.

Ces résultats préliminaires indiquent que l'entelle pourrait servir de modèle acceptable pour la leishmaniose viscérale.

**Références**

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