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Uptake of promastigotes of a lizard *Leishmania* sp. and *Leishmania donovani* by mouse peritoneal macrophages

Short communication

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Introduction

Leishmania are parasitic protozoa. They exist as two well defined forms: the promastigotes in the insect vectors and culture media and the amastigotes in mononuclear phagocytes in mammalian hosts and tissue culture.

Apparently *Leishmania* species that can initiate infections in a given host have developed the ability to resist the effects of activated macrophages of that host, but are readily destroyed by cells of an unsusceptible host. For example, mouse macrophages do not destroy *L. tropica* which infects mice but do kill *L. enriettii*, which fails to infect mice (Mauel et al., 1978). There have been a number of studies on interactions of *Leishmania* from different geographical regions with peritoneal macrophages of different animal species (Chang and Dwyer, 1976; Handman and Spira, 1977). Similar investigations on *Leishmania* isolates from Kenya are scarce in literature. This work was aimed at elucidating more the uptake of promastigotes of lizard *Leishmania* sp. and *Leishmania donovani* by BALB/c macrophages.

Materials and Methods

Two *Leishmania* isolates were used in this study, a lizard and a human strain. The lizard *Leishmania* (Liz. 1, ICIPE 140) was isolated from Kacheliba, Kenya in 1976 through the inoculation of heart blood of a lizard *Mabuya natalensis* into NNN medium. After some unrecorded passages, the promastigotes were frozen as stabilates initially in CO_2 until 1979 when they were transferred to liquid nitrogen.

Leishmania donovani was isolated from a patient in Kenya with a clinical case of kala-azar in 1979. Splenic aspirates were cultured in NNN medium and the promastigotes were stabilated and stored in liquid nitrogen as ICIPE 126 after a number of unrecorded passages.

For these experiments, promastigotes of the two *Leishmania* strains were grown at 25° C in

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Hepes buffered (25 mM) Dulbecco's Modified Eagle Medium (DMEM, Gibco) containing penicillin (100 units/ml), streptomycin (100 μg/ml) and heat inactivated foetal calf serum (FCS, 20% v/v, Gibco). They were harvested at peak growth, washed and counted in a haemocytometer.

Mice of inbread BALB/c strain used, were 7–8 weeks old at the commencement of the experiments. Peritoneal exudate cells were stimulated with 0.5 ml of sterile liquid paraffin. Three days later each mouse was injected intraperitoneally (i.p.) with 2×10^7 promastigotes. The experimental mice were killed at 1 h intervals up to 4 h and at 24 h and their peritoneal cavities washed with 3 ml of DMEM containing heparin (5 units/ml) and antibiotics. Cells collected were pooled accordingly, washed and resuspended in Hepes buffered DMEM containing antibiotics and heat inactivated foetal calf serum. They were added in 0.5 ml volumes to 18×18 mm coverslip in Petri dishes and incubated in a humid atmosphere for 2 h at 37° C. Non adherent macrophages were washed in cold phosphate buffered saline before the coverslips were dried, fixed and Giemsa-stained for light microscopy. The level of infection was determined by counting at least 400 macrophages per coverslip.

Results and Discussion

The human and lizard *Leishmania*/mouse macrophage model system presented here was used to try to simulate the situation in nature where promastigotes of some *Leishmania* species invade macrophages when deposited in the skin of hosts by bites of sandflies and may undergo transformation to amastigotes which either survive or are killed. When 2×10^7 promastigotes were injected intraperitoneally into BALB/c mice, the rate of infection of macrophages was 8–10% $\pm$ 1% for the lizard *Leishmania* sp. and 7–8 $\pm$ 1% for *L. donovani* at 1–4 h, with an average of 2 amastigotes per cell for each of the isolates. In a few cases, mice infected with the lizard isolate had 8–12 parasites per macrophage. Liz. 1 amastigotes were observed to be round or oval in shape. No clear evidence of vacuole development around amastigotes of the lizard isolate was noticed as compared to *L. donovani* where there was some vacuole development (not shown). At 24 h, intact Liz. 1 amastigotes could not be seen but granules and clusters which appeared to be disintegrated parasites were numerous within the macrophages. *L. donovani* amastigotes were, however, clearly visible at this time with distinct outlines and the level of macrophage infection by this parasite isolate was similar to that found after 1–4 h of infection indicating that the parasites had probably not multiplied by this time.

These results suggest that the lizard parasites used in this study could infect warm blooded mammals in nature. But the transient parasitism means that finding the parasites in mammals would be a very rare event. It is common knowledge that prolonged in vitro cultivation of *Leishmania* promastigotes may influence their capability to infect macrophages, a factor which could have had some effects on the two parasite isolates used in this study. But efforts were made to propagate the parasites for the minimal time in vitro and promastigotes were obtained from the original stabilates for new experiments.

Earlier workers have reported similar findings of transient infections after some human volunteers and other lower warm blooded mammals were inocu-

lated with promastigotes of reptilian origin (Manson-Bahr and Heisch, 1961; other literature cited in Belova, 1971). Mutinga and Ngoka (1980) reported apparently fewer cases of human leihmaniasis in certain areas in Kenya where many lizard *Leishmania* isolations had been made than in areas with no lizard *Leishmania*. They hypothetized that *Leishmania* of lizards may play some role in conferring immunity against human infections. But this remains to be thoroughly investigated. The transformation of this lizard *Leishmania* sp. into amastigote forms, in mouse macrophages which normally supports growth of *L. donovani*, is suggestive of some relationship between lizard and true mammalian *Leishmania*. More studies are being conducted in this laboratory to elucidate further the role of reptiles in *Leishmania* epidemiology in Kenya.

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