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In Vitro Infections of Mosquito Cells by Human Parastic Hemoflagellates

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ABSTRACT

The infectivities of four species of human parasitic hemoflagellates to a mosquito ovary cell line, ATO, were studied *in vitro*. Promastigotes of *Leishmania donovani*, *Leishmania major*, and *Leishmania tropica* as well as epimastigotes of *Trypanosoma cruzi* were cultivated with a liquid metacyclic culture (LMC) medium at 27 ℃. Approximatelly 2 x 10^5 ATO cells in 1 ml Hink's insect tissue culture (HITC) medium were seeded onto a 13 mm diameter coverslip in each well of 24-well tissue culture plates. Promastigotes of leishmanias or epimastigotes of trypanosomes were washed and 10^6 organisms in 1 ml HITC medium were added into each well. The coverslips were removed from the well at 24-hr intervals for examination. The percentage of ATO cells infected and intracellular multiplication of the parasite were monitored by examining 3 repeats of 100 randomly selected cells. After 1 day of incubation, all infected ATO cells contained 1 to 2 amastigotes. Number of intracellular parasites increased gradually during the incubation. An average of approximately 5 to 7 amastigotes per infected cell was observed at the end of the third day. The highest percentage of infection was observed in *L. donovani* (2.3%), *L. tropica* (5.7%), or *L. major* (12.3%) at 1, 2, or 3 days after incubation, respectively. Two days later, at most 16 amastigotes could be found in one *L. tropica* infected cells and 4 to 8 in *L. donovani* or *L. major* infected cells. After 3 days of incubation, 32 amastigotes were detected in one *L. major* infected cells. The differences in infectivity and intracellular multiplication in ATO cells among different parasite species needs further investigation.

Key words: *In vitro*, mosquito cell, infectivity, leishmania, trypanosome, amastigote.

Introduction

Parasitic flagellates of the Order Kinetoplastida include the organisms causing African sleeping sickness (*Trypanosoma brucei* spp.), Chagas' disease (*Trypanosoma cruzi*), kala-azar (*Leishmani donovani*), and dermal leishmaniasis (*Leismania* spp.) in humans and many diseases in animals (Vickerman, 1976). Although efforts for the control of these parasitic infections have been tried for more than half a century, these diseases are still the major public health and economical problems in many countries throughout the world (Gibbons, 1992).

Leishmania parasites multiply as promastigotes in the midgut of female phlebotomine sandflies. Infection of a mammalian host occurs when these promastigotes are passed to the host through the mouthparts of the sandflies (Zuckerman and Lainson, 1977). Trypanosomes, such as *T. cruzi*, of the Subgenus *Schizotrypanum* multiply as epimastigotes in the midgut of Reduviid bugs. They have to transform into trypomastigotes to be infective to their hosts. In the vertebrate hosts both leishmanias and *Schizotrypanum* trypanosomes developed intracellularly as amastigotes. An important feature of the pathogenesis in the host is the destruction of macrophages or other infected cells by these amastigotes (Ash, 1991).

Most *in vitro* studies on the interactions between leishmanias and host cells have been performed in the mammalian macrophages (Handman and Goding, 1985; Russell and Wilhelm, 1986; Chao *et al.*, 1991). The entrance of leishmania parasites into macrophages was suggested by phagocytosis rather than by penetration (Lewis and Peters, 1977). In addition to macrophages, many other vertebrate cells could be entried by *T. cruzi*, and many factors have influences on their interaction (Dvorak and Howe, 1976; Liao and Chao, 1986).

Invertebrate cell lines have been established mostly

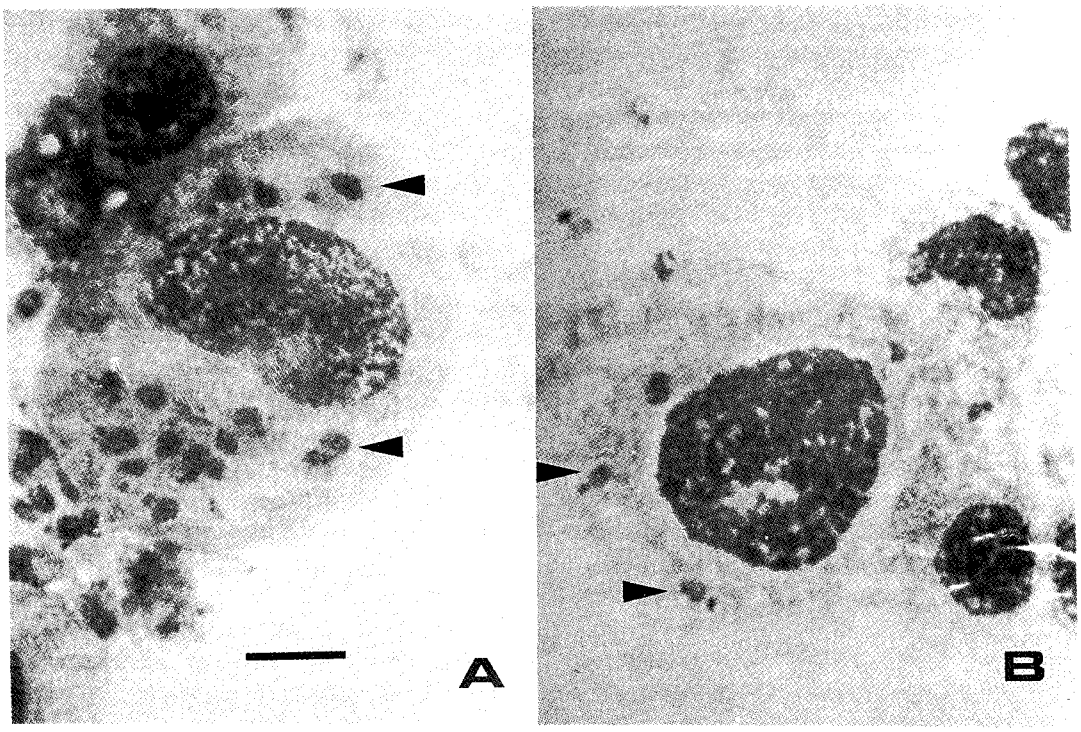

Figure 1. Photomicrograph of infected ATO cells showing intracellular amastigotes (arrow heads) of *Leishmania major* (A) and *Trypanosoma cruzi* (B). Giemsa stained. Scale bar = 10 μm.

for the studies of viruses (Igarashi, 1978; Eckels *et al.*, 1989). Compare to the *in vitro* studies performed in vertebrate cells, the employment of invertebrate cells in the studies of leishamnias and trypanosomes has been hindered. Only triatomine cells have been used in the cultivation of *T. cruzi* (Pudney and Lanar, 1977; Lanar, 1979). In the present study, the infectivities of four species of protozoan, *Leishmania donovani*, *Leishmania major*, and *Leishmania tropica* as well as epimastigotes of *T. cruzi* to a mosquito ovary cell line, ATO, were investigated *in vitro*.

Materials and Methods

Parasites

The parasites used in this investigation were *Leishmania donovani* and *Leishmania tropica* obtained from Dr. W. P. Carney, Naval Bioscience Laboratory, Naval Supply Center, Oakland, CA, U.S.A., *Leishmania major* isolated from a clinical patient with cutaneous lesion in Taipei (Lin *et al.*, 1986), the Tulahuen strain of *Trypanosoma cruzi* obtained from Dr. D. G. Dusanic, Department of Life Sciences, Indiana State University, Terre Haute, IN, U.S.A.. *In vitro* cultures were established as previously described (Chao *et al.*, 1986). Liquid metacyclic culture (LMC) medium (Dusanic, 1980) was used for the weekly passages and for the production of the promastigotes of each species of *Leishmania* and epimastigotes of *T. cruzi* in large quantities. They were cultivated at 27 °C , harvested during the late log phase of growth, washed twice in phosphate buffered-saline (PBS, pH 7.2) by centrifugation at 1,500 *g* for 20 min, and resuspended in LMC medium to a concentration of 1×10^6 organisms/ml until use.

ATO cells

The invertebrate cell line employed in this study was originally isolated from the ovary of a normal female mosquito, *Aedes togoi*. It was obtained from Dr. C. M. Ho, Department of Parasitology, National Yangming Medical College, Taipei and has been maintained in Hink's insect tissue culture (HITC) medium containing 10% FCS in tissue culture flasks. Approximatelly 2×10^5 ATO cells in 1 ml HITC medium were seeded onto a 13 mm diameter coverslip in each well of 24-well tissue culture plates. They were incubated at 27 °C overnight to allow the cells to settle and adhere to the cover slips. Non-adherent cells were removed by dipping the cover slips into HITC medium

Table 1. *In vitro* infectivities of human parasitic hemoflagellates in mosquito ATO cells

Parasite species	Percentages of cells infected after		
	1d	2d	3d
L. donovani	2.3	4	8.3
L. major	1.0	2	12.3
L. tropica	1.0	5.7	4.3
T. cruzi	1.3	2.3	7.0

three times. The coverslips with adherent cells were then transferred to new plates with fresh culture medium for infectivity tests.

Infectivity test

Motile leishmanial promastigotes or trypanosomal epimastigotes were washed as described above and 10^6 organisms in 1 ml medium were added into each well of ATO cell cultures. The coverslips were removed from the well at 24-hr intervals for examination. They were washed by dipping the cover slips into PBS (pH 7.2) three times, air dried, fixed with 100% methanol, and stained with Giemsa solution. The percentages of ATO cells infected by each species of these parasites were examined by observing 3 repeats of 100 randomly selected cells. Intracellular multiplication of parasites was monitored for 3 days by averaging the numbers of amastigotes found in parasite-containing cells of each group.

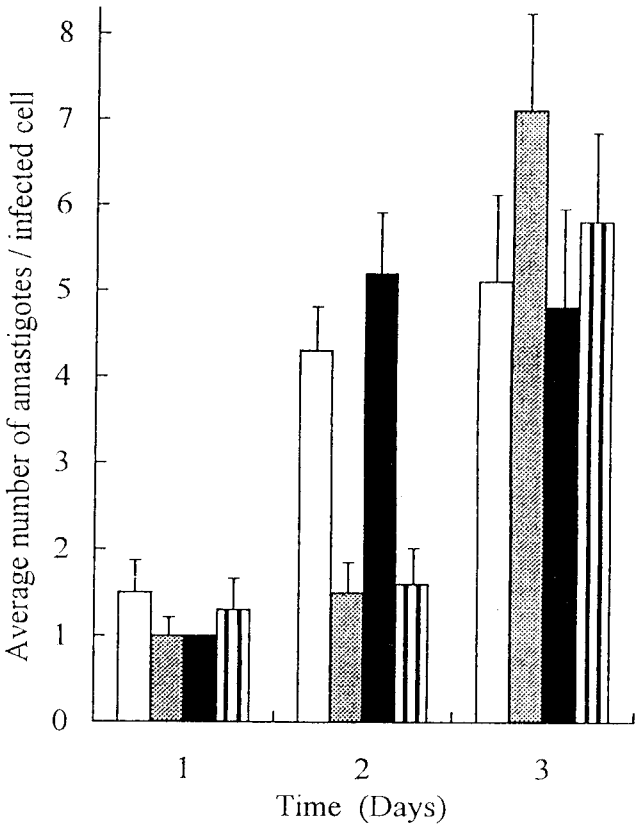

Figure 2. Intracellular multiplication of human parasitic flagellates in mosquito cells. Open bar, *L. donovani*; Shadowed bar, *L. major*; Dark bar, *L. tropica*; straited bar, *T. cruzi*. Average numbers of intracellular parasites per infected cell are presented as Mean ± S.E.; n = 3.

Results

Both promastigote stage of leishmanias and epimastigote stage of trypanosomes exhibited infectivity for ATO cells. Figure 1 shows that the infection with either stage resulted the development of intracellular amastigotes. The ATO cells were thus considered susceptable to all four different species of hemoflagellates examined.

The percentages of infected ATO cells increased along with incubation except a decline was detected in cells reacted with *L. tropica* on the third day (Table 1). The highest percentage of cell infection was observed in *L. major* (12.3%) at three days after incubation. While at one or two days after incubation, the highest percentage was found in *L. donovani* (2.3%) or in *L. tropica* (5.7%), respectively. The percentages increased and reached to 8.3 and 7.0 at the end of three days of infection in *L. donovani* and in *T. cruzi*, respectively.

Figure 2 shows intracellular multiplication of these flagellages in ATO cells. All infected cells contained 1 to 2 amastigotes initially, no matter of leishmanial or trypanosomal origin. Average numbers of intracellular organisms per infected cell increased gradually during the incubation except the number in *L. tropica*-infected cells maintained at the same level on the third day.

After two days of incubation, at most 16 amastigotes could be found in one of the *L. tropica*-infected cell, 8 and 4 in *L. donovani*-and *L. major*-infected cells, respectively. Whereas at the same time only 1 or 2 amastigotes could be observed in *T. cruzi*-infected cells. The average number of parasites in *L. donovani*- or *L. tropica*-infected cell was significantly higher than that in *L. major*- or *T. cruzi*-infected cell. An average around 5 to 7 amastigotes per infected cell was observed at the end of the third day. An *L. major*-infected cell harbored the highest number (32) of intracellular organisms was discovered three days after incubation.

Discussion

The fates of intracellular parasites varies from species to species and from stages to stages. They enter cells either via penetration actively or via phagocytosis passively. Intracellular multiplication was usually found only within permissive cells. The rate of cell invasion and infection is affected by different characteristics of each species and genus of parasites, the ratio of parasite to cell, the temperature, the pH, and the cell-parasite contact time (Alcantara and Brener, 1978). The surface membrane of a parasite also plays a significant role in infection. All interactions between host and parasite occur at least initially at the level of the parasite's surface membrane. Membrane-bound lectins appear to play a role in intercellular recognition and may serve as important probes for analyses of the properties of cell surface components (Chao *et al.*, 1990).

The results in this study indicated that ATO cells were entried and employed as multiplication habitates by all four trypanosomatid species tested. The ATO cell was thus considered susceptible to all four different species of hemoflagellates examined. Application of ATO cells has permitted the production of sufficient numbers of parasites for biochemical and immunological investigations in future. Schaub (1994) compared the *in vivo* pathogenicity of 18 different species of trypanosomatids on insects. Obviously, *in vitro* studies correlate with *in vivo* observations should be a more interest thing to do, though there is no *in vivo* indications that these parasites enters any of their vector cells. Growth and differentiation of *T. cruzi* has been studied on an embryo cell line of its host *Triatoma infestans* (Lanar, 1979). However, susceptibilities of this vector cell line for different trypanosomatids were not studied yet. ATO cell has the potential to be developed as a standard cell line for the study of vector relationships in Trypanosomatidae. The differences in intracellular multiplication patterns in ATO cells among different species of hemoflagellates are worth further investigation.

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人體血液及組織寄生之鞭毛蟲對蚊子細胞的體外感染

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摘 要

四種非斑蚊媒介之人體血液及組織寄生鞭毛蟲：*Leishmania donovani*, *Leishmania major*, *Leishmania tropica*, 及*Trypanosoma cruzi*, 分別以液態的 LMC 培養液在 27℃ 培養。另由斑蚊所分離的卵巢 (ATO) 細胞，以 Hink's insect tissue culture (HITC) 培養液 在 27℃培養。以血球計數器算細胞數目後調整濃度為每毫升含 2×10^5 個 ATO 細胞，以每槽一毫升的量，分別加入 24 槽細胞培養盤中，每槽中預置有適當大小的圓形蓋玻片一片，27℃ 隔夜培養後在各槽中分別加入 1 毫升內含 10^6 上述各種鞭毛蟲的LMC培養液，再置於 27℃ 培養，每 24 小時取出一批上有細胞的蓋玻片，風乾後，以絕對甲醇固定，經姬姆薩氏染液染色並水洗風乾後，倒封在載玻片上，以顯微鏡觀察結果。一天後受感染的 ATO 細胞於細胞質中出現 1 至 2 個無鞭型 (amastigote) 蟲體；二天後受 *L. tropica* 感染之細胞出現 16 個無鞭型蟲體，*L. donovani* 出現 4 至 8 個，而其他則仍為 1-2 個；三天後，*L. major* 感染出現 32 個無鞭型蟲體，而其他則為 1 至 16 個不等的無鞭型蟲體。除了錐鼻蟲的 haemocytes 之外，本篇為首次報告人體血液及組織寄生鞭毛蟲在體外培養無脊椎動物細胞內的分裂及發育。

關鍵詞：體外培養、蚊蟲細胞、感染性、利什曼原蟲、錐蟲、無鞭型蟲體