Comparative positions of kinetoplasts in *Trypanosoma musculi* and *Trypanosoma lewisi* during development *in vitro*

M. Ashraf, R. A. Nesbitt, P. A. Humphrey, M. Siewe and C. M. Lee

Department of Biology, Howard University, Washington DC, USA

Received 10 August 2001; revision accepted 23 January 2002

Abstract. The development of *Trypanosoma musculi* and *Trypanosoma lewisi* were studied *in vitro* in the presence of adherent splenic cells. Both parasites developed only when attached by their flagellar tips to adherent splenic cells. During the proliferation of *T. musculi*, the kinetoplast migrated towards the nucleus, and once in the vicinity of the nucleus, the nuclear division was triggered. The kinetoplast of *T. lewisi* did not migrate towards the nucleus, but remained at its original location. The nucleus and kinetoplast divided at the same time in both parasites, and parasites started dividing from their flagellar ends and *T. musculi* and *T. lewisi* daughter cells were formed within 48 h. The unavailability of the adherent splenic cells *in vitro* led the parasites to transform into round/oval nonviable forms.

INTRODUCTION

Trypanosoma musculi is parasitic to mice and *Trypanosoma lewisi* is parasitic to rats. Both of these parasites are highly specific to their hosts and are considered to have two developmental stages in their vertebrate hosts, an epimastigote form in which kinetoplast is anterior to nucleus, and a trypomastigote form in which kinetoplast is posterior to nucleus. The infective metacyclic forms are transmitted to their hosts via faeces of insect vectors, during grooming and licking (Minchin & Thomson 1915; Hoare 1964, 1972). The inoculated metacyclic trypomastigotes transform into epimastigotes, which then undergo proliferation and live in the bloodstream extracellularly, mostly as trypomastigotes. These parasitic infections terminate naturally 24–28 days postinoculation (Hogan & Lee 1987; Humphrey *et al.* 1997).

Trypanosoma musculi and *T. lewisi* belong to the Kinetoplastida, which is characterized by the presence of kinetoplast, a specialized DNA-containing region of the single mitochondrion, and the mitochondrial DNA of kinetoplast is known as kinetoplast DNA (kDNA). Kinetoplast DNA is an organization of concatenated maxicircles and minicircles; the maxicircles have genetic function similar to mitochondrial DNA, and the minicircles are involved in RNA editing (Simpson 1987; Shapiro & Englund 1995). Positioning of the kinetoplast during cell division plays an important role in proper replication of concatenated DNA circles (Lun & Dessler 1995; Matthews *et al.* 1995).

Trypanosoma musculi and *T. lewisi* parasites have been cultivated in the presence of adherent cells *in vitro* (Albright & Albright 1980; Vincendeau *et al.* 1986; Smith *et al.* 1987; Mohamed

et al. 1988; Ashraf *et al.* 1999). Both parasites, in the absence of adherent cells, were unable to grow for more than 6–8 days *in vitro*.

The present research was conducted to examine the differences in development of *T. musculi* and *T. lewisi* and the positioning of the kinetoplast during cell division *in vitro*.

MATERIALS AND METHODS

Parasites

Trypanosoma musculi (isolate #30182; Lincicome) and *T. lewisi* (New Orleans-67) were obtained from American Type Culture Collection; ATCC, Manassas VA. The parasites were maintained *in vivo* by inoculating mice or rats. The trypanosomes were collected from heparinized blood, which was drawn by cardiac puncture of infected animals. The blood was centrifuged at 1300 *g* for 20 min, and the pellet containing parasites was resuspended in 40 ml of Dulbecco's Phosphate Buffered salt solution (DPBS). The cell suspension was centrifuged at 300 *g* for 10 min to remove blood cells, and supernatant containing the parasites was centrifuged at 1300 *g* for 20 min. The pellet, obtained from the centrifugation, was washed 4 times with DPBS by centrifugation at 1300 *g* for 20 min. The parasites were counted using a haemocytometer. Experimental animals were inoculated intraperitoneally with 0.25 ml of DPBS containing 1×10^3 trypanosomes. These parasites were also maintained *in vitro* by culturing in the presence of adherent splenic cells in a 5% CO₂ humidified chamber at 37 °C (Ashraf *et al.* 1999).

Isolation of adherent splenic cells

Adherent splenic cells were obtained from Balb/C female mice (Charles River, Kingston, NY) and N:S/D Sprague-Dawley female rats (NIH, National Center of Research Resources, Bethesda, MD). Spleens were removed aseptically, placed in 3 ml of RPMI medium 1640 (GibcoBrl/Invitrogen, Grand Island NY), and teased apart with forceps. To disperse the cells further, the suspension was drawn into a 3-ml syringe, through a 23-gauge needle, and discharged through a 26-gauge needle into a 15-ml tube containing 10 ml of RPMI medium 1640. This cell suspension was washed 3 times in the same medium by centrifugation at 300 *g* for 10 min. After the final wash, the spleen cells were cultured using RPMI medium 1640 supplemented with 10% fetal bovine serum and 100 units/ml of penicillin G and streptomycin sulphate (complete medium) in a 5% CO₂ humidified chamber at 37 °C. The spleen cells were cultured either in 25 cm² tissue culture flasks (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ) or tissue culture chamber slides (Laboratory-Tek Products, Naperville, IL). After 48–72 h of incubation, nonadherent cells were removed by changing the medium twice. Any remaining floating cells were removed in the next 24 h. Based on cell structure and presence of collagen (electron micrographs), fibroblasts were identified as a major cell population in adherent cells. The percentage of macrophages in adherent cells was not calculated. Both macrophages and fibroblasts supported the growth of *T. musculi* and *T. lewisi* (Albright & Albright 1980; Mohamed *et al.* 1988).

Cell cultures

Adherent splenic cells (5×10^6) were grown in 0.5 ml of complete medium using 8 tissue-culture chamber slides. The adherent splenic cells formed a monolayer and all nonadherent cells were removed by changing the culture medium after 48–72 h of incubation.

Trypanosoma musculi and *T. lewisi* (500 parasites) were isolated from the blood of infected animals, suspended in 0.5 ml of complete medium and added to each chamber containing adherent cells of mice or rats, respectively. The slide chambers containing the adherent cells and the parasites

were cultured in a 5% CO₂ humidified chamber at 37 °C. The slide chambers were removed at intervals of 24, 48, 72, and 96 h. At each interval, the medium was removed and the chambers were air-dried, stained with Diff-Quik (American Scientific Products, McGraw Park, IL), and photographed at 1000× under oil immersion.

RESULTS

Trypanosoma musculi and *T. lewisi* parasites were observed to attach to the adherent splenic cells by their flagellar tips prior to proliferation. Within 24 h of attachment, the kinetoplast of *T. musculi* migrated towards the nucleus, and the parasite started dividing (binary fission) from the flagellar end into two (Figs 1b and c and 2a and b). Each time, when kinetoplast approached the nucleus, nuclear and kinetoplast divisions were initiated. Both daughter nuclei and kinetoplasts remained close together (forming a pair) during cell division. After the cytoplasmic division was completed, the kinetoplast in each daughter parasite started moving towards the posterior end, and trypomastigote forms were produced within 48 h. The newly developed trypomastigotes remained active, floating up to 4 days. During proliferation *T. musculi* parasites transformed into a shorter form, and dividing parasites formed a rosette-like pattern around the adherent cells.

Unlike *T. musculi*, the kinetoplast of *T. lewisi* did not migrate towards the nucleus, but stayed at its original location and divided as a trypomastigote (Fig. 2). During cell division both nucleus

Figure 1. Migration of kinetoplast towards the nucleus in *Trypanosoma musculi* during *in vitro* development. The figure shows (a) kinetoplast original location; (b) once kinetoplast is close to nucleus, both nucleus and kinetoplast divide; (c) division of cytoplasm. Insert (Tm) in upper right corner shows nonviable forms of *T. musculi*. Kinetoplast is clearly visible. Scale bar = 10 µm; Insert is magnified 2×.

Figure 2. Position of kinetoplast during *in vitro* development of *Trypanosoma lewisi*. The figure shows (a) division of kinetoplast independently of nucleus and it stays at its original location (posterior end of cell); (b) division of cytoplasm. Insert (L) shows nonviable form *T. lewisi*. Both kinetoplast and nucleus are visible. Scale bar = 10 μ m.

and kinetoplast divided at the same time, and the parasite divided from the flagellar end. Like *T. muscui*, *T. lewisi* daughter cells were formed within 48 h. *Trypanosoma lewisi* did not form rosette-like pattern around the adherent cells and remained as trypomastigote forms. It was also observed that after 48 h in culture *T. lewisi* trypomastigotes were able to divide without attaching to adherent cells.

Both parasites were maintained in culture for more than 12 months by transferring the cultured parasites to new adherent splenic cells at intervals of 4–6 days. In the absence of adherent cells, both parasites were unable to proliferate and were transformed into motionless round/oval forms. Trypan blue staining indicated that the parasites in this form were nonviable. In nonviable form of *T. muscui* the kinetoplast is clearly visible while it seems that nuclear material is disintegrated; however, in *T. lewisi* both the kinetoplast and nucleus are visible (Figs 1 and 2, inserts). The addition of new adherent splenic cells did not revive the motionless parasites and these parasites were unable to infect the host.

DISCUSSION

The structural changes of trypanosomes in the absence of interaction of trypanosomes with adherent splenic cells, suggests that *T. muscui* and *T. lewisi* could be undergoing programmed cell death (Welburn *et al.* 1997).

It is known that *T. musculi* and *T. lewisi* are host specific; however, it is still not known what particular criteria make them host specific. It has been shown that kDNA minicircles are heterogeneous in different species and isolates of same species of trypanosomes. Kinetoplast DNA may play an important role in speciation of these trypanosomes. Also the positioning of the kinetoplast during cell division may play an important role in proper replication of kDNA minicircles which have conserved regions for different species of trypanosomes for successful morphological transformation of trypanosomatids (Morel *et al.* 1980; Kidane *et al.* 1984; Jasmer & Stuart 1986; Ray 1989).

The present study indicated that *T. musculi* and *T. lewisi* require the attachment to the adherent splenic cells for their development and proliferation. During *in vitro* development, it was observed that *T. lewisi* divided as trypomastigotes and their kinetoplast did not migrate towards the nucleus. *Trypanosoma musculi* was distinctive in that it transformed into a stumpy structure and kinetoplast moved closer to the nucleus (epimastigote forms) before dividing, and then moved posterior to the nucleus.

REFERENCES

- ALBRIGHT JW, ALBRIGHT JF (1980) *In vitro* growth of *Trypanosoma musculi* in cell-free medium conditioned by rodent macrophages and mercaptoethanol. *Internatl J. Parasitol.* **10**, 137.
- ASHRAF M, NESBITT RA, GUGSSA A, LEE CM (1999) Development and proliferation of *Trypanosoma musculi* in the presence of adherent splenic cells. *J. Parasitol.* **85**, 129.
- HOARE CA (1964) Morphological and taxonomic studies on the mammalian trypanosomes. *X. Revision Syst. J. Protozool.* **11**, 200.
- HOARE CA (1972) *The trypanosomes of mammals. A zoological monograph*. Oxford, UK: Blackwell Scientific Publications.
- HOGAN Y, LEE CM (1987) Interaction of trace metal contaminants on hematological responses: influence of infection with *Trypanosoma lewisi*. *J. Nat. Med. Assoc.* **79**, 521.
- HUMPHREY PA, ASHRAF M, LEE CM (1997) Growth of trypanosomes *in vivo*, host body weight gains, and food consumption in zinc deficient mice. *J. Nat. Med. Assoc.* **89**, 48.
- JASMER DP, STUART K (1986) Conservation of kinetoplastid minicircle characteristics without nucleotided sequence conservation. *Molecul. Biochem. Parasitol.* **18**, 257.
- KIDANE GZ, HUGHES D, SIMPSON L (1984) Sequence heterogeneity and anomalous electrophoretic mobility of kinetoplast minicircle DNA from *Leishmania tarentolae*. *Gene* **27**, 265.
- LUN ZR, DESSER SS (1995) Is the broad range of hosts and geographical distribution of *Trypanosoma evasi* attributable to the loss of maxicircles kinetoplast DNA? *Parasitol. Today* **11**, 131.
- MATTHEWS KR, SHERWIN T, GULL K (1995) Mitochondrial genome repositioning during the differentiation of the African trypanosome between life cycle forms is microtubule mediated. *J. Cell Sci.* **108**, 2231.
- MINCHIN EA, THOMSON JD (1915) The rat-trypanosome, *Trypanosoma lewisi*, in its relation to the rat-flea, *Ceratophyllus fasciatus*. *Quart. J. Microscop. Sci.* **60**, 463.
- MOHAMED HA, MARAGI S, WALLBANKS KR, MOLYNEUX DH (1988) *In vitro* cultivation of herpetosoma trypanosomes on embryonic fibroblast and in semidefined cell-free medium. *J. Parasitol.* **74**, 421.
- MOREL C, CHIARI E, CAMARGO EP, MATTEI DM, ROMANHA AJ, SIMPSON L (1980) Strains and clones of *Trypanosoma cruzi* can be characterized by pattern of restriction endonuclease products of kinetoplast DNA minicircles. *Proc. Natl Acad. Sci.* **77**, 6810.
- RAY DS (1989) Conserved sequence blocks in kinetoplast minicircles from diverse species of trypanosomes. *Molecul. Cell. Biol.* **9**, 1365.
- SHAPIRO TA, ENGLUND PT (1995) The structure and replication of kinetoplast DNA. *Ann. Rev. Microbiol.* **49**, 117.
- SIMPSON L (1987) The mitochondrial genome of kinetoplastid protozoa: genomic organization, transcription replication, and evolution. *Ann. Rev. Microbiol.* **41**, 363.
- SMITH KS, ALBRIGHT JW, ALBRIGHT JF (1987) The limitation of growth of *Trypanosoma musculi* *in vitro*. *Internatl J. Parasitol.* **17**, 1435.
- VINCENDEAU P, GUILLEMAN B, DAULOUEDE S, RIPERT C (1986) *In vitro* growth of *Trypanosoma musculi*: requirements of cells and serum free culture medium. *Internat. J. Parasitol.* **16**, 387.
- WELBURN SC, BARCINSKI MA, WILLIAMS GT (1997) Programmed cell death in trypanosomatids. *Parasitol. Today* **13**, 22.