Short Report

Importance of acidic intracellular compartments in the lysis of *Trypanosoma brucei brucei* by normal human serum

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The unicellular parasite *Trypanosoma brucei brucei* is unable to infect healthy humans due to its sensitivity to a component of normal human serum (NHS), high density lipoprotein (HDL) (RIFKIN, 1978; LORENZ *et al.*, 1994). Unable to synthesize essential lipids *de novo*, *T.b. brucei* bloodstream forms are obliged to assimilate these compounds from their host. HDL and low density lipoproteins (LDL) can supply the required lipids (BLACK & VANDEWEERD, 1989) and high affinity receptors for both lipoproteins are present on the parasites (COPPENS *et al.*, 1988; GILLETT & OWEN, 1992). Whereas LDL is known to bind to the trypanosomal cell surface in the area of the flagellar pocket followed by internalization and delivery to endosome/lysosome-like compartments (COPPENS *et al.*, 1987), the interaction between *T.b. brucei* and human HDL and the molecular events leading to cell lysis are not well understood. Clearly, after a lag phase of 20–30 min at 37°C in medium containing NHS, the plasma membrane of the trypanosomes is damaged resulting in increased permeability, osmotic swelling and lysis (RIFKIN, 1984).

We present evidence that acidic intracellular compartments known to be involved in processes such as receptor recycling, membrane traffic, degradation of endocytosed macromolecules, and toxin activation in mammalian cells (MELLMAN *et al.*, 1986) and, importantly, in the LDL metabolism of *T. b. brucei* (see COPPENS *et al.*, 1993) are also involved in HDL-mediated trypanolysis. The lysosomotropic weak bases ammonium chloride (NH_4Cl) and chloroquine (CQ) and the carboxylic ionophore monensin, which raise the pH in intracellular compartments and inhibit the above processes, completely inhibited trypanolysis in an assay *in vitro* (Fig. 1). *T. b. brucei* could even be cultivated *in vitro* for 4 d on a *Microtus montanus* embryo fibroblast feeder layer in the presence of 20% NHS and 20 mM NH_4Cl (data not shown). Furthermore, it seems that, in analogy with LDL (COPPENS *et al.*, 1988), gold-coupled human HDL_3 is internalized by *T. b. brucei* and directed to lysosomal compartments (Fig. 2, A). The uptake is not abrogated, but only marginally slowed, if NH_4Cl is present (Fig. 2, B and other data not shown). Which of the pH-dependent processes listed above is required for trypanolysis is unclear. The observation that trypanolysis does not occur, despite HDL uptake, indicates that additional pH-dependent processes (e.g. toxin activation or metabolic degradation) may be required to cause cell lysis. The data presented are consistent with a model of trypanolysis suggested by BETSCHART *et al.* (1989) and elaborated by HAJDUK *et al.* (1992), involving binding of try-

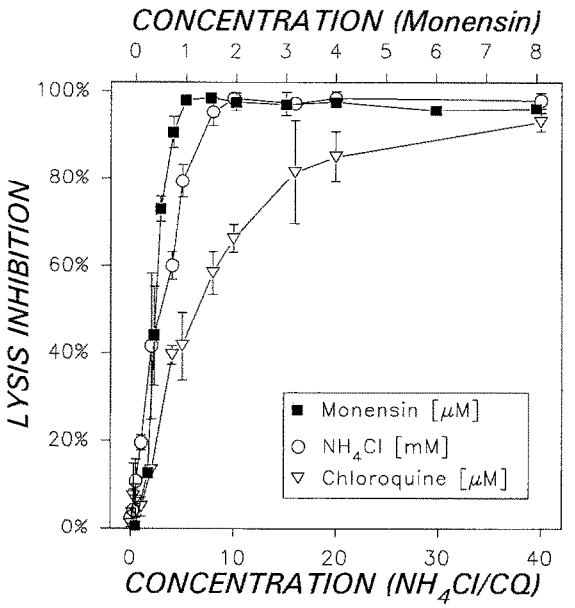

Fig. 1. Inhibition of trypanolysis by normal human serum (NHS). *T. b. brucei* (STIB 345AD) was isolated 72 h after infection of ICR mice, incubated *in vitro* at 37°C in minimal essential medium (MEM) containing 30% NHS, 10% horse serum (LORENZ *et al.*, 1994) plus various concentrations of NH_4Cl (Merck), chloroquine (Sigma) or monensin (Sigma). After 4 h (monensin 3·3 h), lysis was calculated by determining the proportion of swollen/lysed cells in the population using phase-contrast microscopy (200× magnification). Lysis was normalized to give 100% for the highest observed value and lysis inhibition (%) was calculated by subtracting the normalized values from 100%. Values are means of duplicate or triplicate assays (vertical bars indicate standard deviation, ≥100 cells counted per incubation).

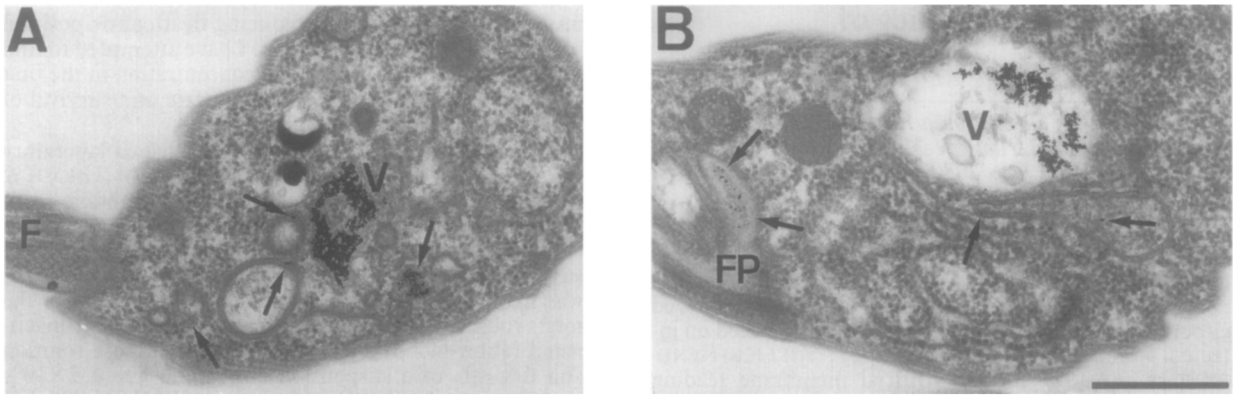

Fig. 2. Effect of NH_4Cl on uptake of high density lipoproteins (HDL) by *T. b. brucei*. Trypanosomes (prepared as described in Fig. 1) were incubated for 60 min at 37°C in MEM (plus 5 mM EDTA, without Ca^{2+}/Mg^{2+}/phosphate), 1% bovine serum albumin, 50 μg/mL human HDL_3–gold (10 nm gold particles) (HDL provided by Dr J. S. Owen) without (A) or with (B) 20 mM NH_4Cl and processed for electron microscopy. A. Endocytosed HDL_3–gold particles are found within tubulovesicular structures (arrows) and amassed in large vacuolar structures. B. The same endocytic organelles are labelled in the presence of NH_4Cl, but the large vacuoles are dilated in a fashion characteristic of acidic compartments (e.g. lysosomes; COPPENS *et al.*, 1993). F, flagellum; FP, flagellar pocket; V, vacuole/lysosome. Scale bar 0·5 μm.

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panolytic HDL to cell surface receptors followed by internalization and delivery to acidic intracellular compartments.

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Received 17 March 1994; accepted for publication 17 May 1994

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (1994) **88**, 488–489

Short Report

A modified artificial membrane feeding method for the study of the transmission dynamics of leishmaniasis

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Study of the transmission dynamics of leishmaniasis has recently undergone a resurgence of interest. Experimental infection of sandflies is usually achieved either by allowing laboratory-bred sandflies to feed on infected animals (SHORTT *et al.*, 1931; KILLICK-KENDRICK *et al.*, 1977) or by artificial membrane feeding methods (ADLER & BER, 1941; POZIO *et al.*, 1985). The difference between the 2 approaches is that in the latter the parasite concentration in the infective meal can be controlled, while in the former parasite abundance in the skin is comparatively low and, in the case of visceral leishmaniasis, even the presence of parasites is unpredictable.

Giving a second blood meal to sandflies is always a problem as most of them die at oviposition and a second feed before oviposition is not always possible as most of the flies are gonotrophically concordant (KILLICK-KENDRICK, 1987). SHORTT *et al.* (1926), working with *Phlebotomus argentipes*, a gonotrophically concordant fly, investigated many ways of reducing death at oviposition but without encouraging results. I have attempted to find out whether reducing the blood concentration in the first feed had any effect on the infection rate and survival of sandflies.

The sandflies used were from a established laboratory colony of *P. argentipes* Annandale & Brunetti (GHOSH & BHATTACHARYA, 1989). Details of the membrane feeding technique have been described by GHOSH *et al.* (1989), except that the constitution of the blood–parasite suspension was varied. The suspension (about 2·3 mL) was made up of about 0·7 mL (almost one-third of the total volume) of defibrinated and complement-inactivated rabbit blood, 0·8 mL of 0·254 M sucrose solution and 0·8 mL of a suspension containing about 2×10^6–4×10^6/mL of *Leishmania donovani* (MHOM/IN/90/GE6) (amastigotes in group A experiments, promastigotes in group B) in phosphate-buffered saline solution (PBS) at pH 7·2. The promastigotes were grown in modified biphasic Tobie's medium (TOBIE *et al.*, 1950), while macerated spleen of infected Balb/c mice served as the source of amastigotes. As a control, a proportion of the flies

Table. Results of membrane feeding and subsequent animal feeding of *Phlebotomus argentipes*

Group[a]	First blood meal[b]	No. dying during Oviposition	No. dying during Maintenance	Second blood meal[b]	Numbers Dissected	Infected[c]	Dying during oviposition	Lost during maintenance	Third blood meal[b]
A	207/246	–	28	144/179	36	27	69	15	34/59
B	221/269	–	35	149/186	36	21	71	16	37/63
C	251/300	143	40	56/68	20	12	48	–	–

[a]Each group represents the total of 4 trials.
[b]No. feeding/no. offered food. First blood meal contained *Leishmania* in membrane feeding apparatus; second and third meals were on uninfected hamsters or mice.
[c]Containing *Leishmania* promastigotes.