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Separation of *Trypanosoma cruzi*-infected from non-infected P815 cells

Short communication

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A variety of cell culture systems have been used to establish in vitro *Trypanosoma cruzi* infection (reviewed by Brener, 1973). Unlike amastigote forms of several species of *Leishmania*, which have been shown to grow in non-adherent as well as adherent cells (Berens and Marr, 1979; Berman et al., 1979; Nacy and Diggs, 1981), cells used to produce *T. cruzi* infection have been those producing adherent monolayers such as, for example, fibroblasts, myoblasts and macrophages. One inherent difficulty of working with adherent cell types is the need for either enzymatic or other chemical treatments for detachment of the cells from each other and from the walls of tissue culture flasks when subsequent work is to be done with the infected cells. Alterations produced during those treatments may result in the production of artifacts or misleading results. Another common difficulty encountered while working with *T. cruzi*-infected cell cultures is the relatively low proportion of cells which harbor the parasite at any given time. Thus, biochemical changes induced by the presence of *T. cruzi* may go unnoticed against the relatively large background provided by the uninfected cells. In this note, we report the successful infection of a non-adherent cell line and a procedure for preparing suspensions containing high proportions of *T. cruzi*-containing cells.

Tulahuén strain trypomastigote forms of *T. cruzi* used in this work were maintained by serial intraperitoneal (i.p.) passages in 4-week-old CD_1 mice (Charles River, Wilmington, Massachusetts). Circulating parasites were aseptically purified by centrifugation of infected mouse blood collected in heparinized tubes over Lymphoprep (specific gravity 1.077, Nyegaard, Oslo) (Budzko and Kierszenbaum, 1974) followed by chromatography on diethylaminoethyl-cellulose (Mercado and Katusha, 1979). The P815 mastocytoma cell line, originally derived from a methylcholantrene-induced tumor of DBA/2 mice, was

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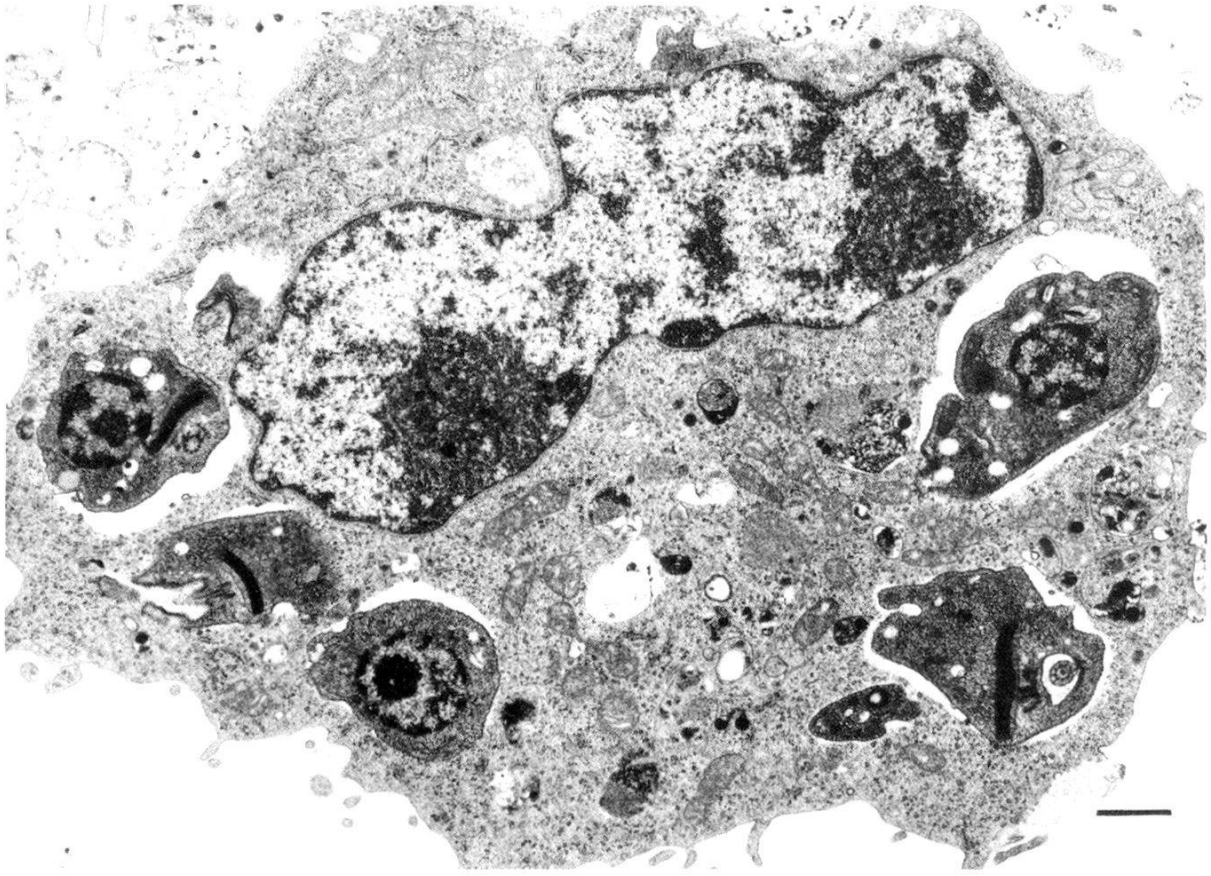

Fig. 1. P815 cell containing amastigote forms of *T. cruzi*. Bar = 1 μm.

maintained by transfers in Dulbecco's modified Eagle's minimal essential medium (KC Biological, Lenexa, KS) containing 10% heat-inactivated fetal calf serum (Microbiological Associates, Walkersville, Maryland), 100 units/ml penicillin and 100 μg/ml streptomycin (D-MEM) and grown at 37° C in a 5% CO_2-in-air atmosphere saturated with water vapor.

In a typical in vitro infection experiment, 0.1 ml of a suspension of *T. cruzi* trypomastigotes in D-MEM (at 5×10^8 organisms/ml) was used to infect 4.9 ml of a suspension of P815 cells in the same medium (at 1.02×10^6 cells/ml) to provide a parasite : cell ratio of 10 : 1. After incubation at 37° C for 2 days in the presence of 5% CO_2, the cultures were centrifuged at 130 *g* for 5 min at 4° C. The cells in the pellet were resuspended in D-MEM and centrifuged again under the same conditions. These two steps resulted in an almost complete removal of the free-swimming parasites. The P815 cells were then incubated in D-MEM medium for an additional 24 h, washed twice in Tyrode's salt solution by centrifugation and resuspended in the same solution. At this point, approximately 30% of the P815 cells were infected as determined by microscopic screening of smears fixed with 2.5% glutaraldehyde and stained with Giemsa. Cells from infected and uninfected cultures were processed for electron microscopy as described previously (Villalta et al., 1979). Shown in Fig. 1 are a number of amastigote forms of *T. cruzi* within an infected P815 cell.

Infected P815 cell cultures were centrifuged at 450 *g* and the cells washed twice with Tyrode's solution. After resuspension in the same solution, the cells were carefully layered over a discontinuous density gradient consisting of 2 ml solutions of metrizamide (Nyegaard) of specific gravity 1.120 (bottom layer), 1.100 and 1.090 set up in a 15 ml plastic tube (Catalog No. 25310, Corning, Corning, New York). Centrifugation conditions were 450 *g* for 15 min at 4° C. The cell-containing fractions were recovered; these were the pellet, which consisted of approximately 80% infected cells, and two other fractions found on top of the 1.100 and 1.090 layers, which consisted of mostly uninfected cells. When infected cell suspensions devoid of free-swimming parasites were further incubated in D-MEM, trypomastigotes were detected in the medium after 4 days. These trypomastigotes readily infected fresh cultures of P815 cells.

Infection by *T. cruzi* of suspended cell cultures, coupled with the possibility of separating the infected from most of the uninfected cells, precludes the use of enzymatic or chemical treatments for retrieval of the cells and greatly reduces the possibility that uninfected cells contribute to obscure important events taking place in the infected cells at a higher or lower rate. The P815 cell line has been frequently used by immunologists as a target cell model in studies of the mechanisms of cell-mediated lysis. In view of the present report of infection of these cells with *T. cruzi*, already available techniques could be readily applied to examining the contribution of the intracellular infection to development of sensitivity to immunological destruction. Furthermore, infected P815 cells could be used to simplify the preliminary screening of drugs with selective toxicity for the intracellular form of the parasite.

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