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Cystoisospora belli: In vitro multiplication in mammalian cells

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Abstract

Intracellular development of *Cystoisospora belli* was demonstrated in 4 different mammalian cell lines. Human ileocecal adenocarcinoma (HCT-8), epithelial carcinoma of lung (A549), Madin-Darby bovine kidney (MDBK), and African green monkey kidney (VERO) were exposed in vitro to *C. belli* sporozoites, which had been isolated from the feces of HIV-AIDS patients. Parasites invaded all the cellular types between 4 and 12 h after exposure and multiplication was demonstrated after 24 h. Grater number of merozoites formed in VERO cells, followed by HCT-8. In the MDBK and HCT-8 cells, the parasitophorous vacuole was less evident and immobile merozoites were observed in the cytoplasm. In VERO cells, one or several parasitophorous vacuoles contained up to 16 mobile sporozoites. No oocysts were found in any of the cell types used. VERO cells may be suitable for studies of the interaction between parasite and host cells.

Index Descriptors and Abbreviations: *Cystoisospora belli*; Cell culture; In vitro multiplication; *Isospora belli*

1. Introduction

The genus *Cystoisospora* was proposed because of the presence of unizoic tissue cysts in lymphoid tissues in rodents which function as intermediate hosts of *Isospora felis* and *Isospora rivolta* of cats (Frenkel, 1977). After a review of molecular affinities of many *Isospora* species from birds and mammals, Barta et al. (2005) assign all tetrasporozoic, diplosporocystic oocysts from mammals without Stieda bodies in their sporocysts, to the genus *Cystoisospora* (Sarcocystidade), and all such oocysts from birds with Stieda bodies in their sporocysts to the genus *Isospora*.

Cystoisospora belli is an obligate intracellular protozoan, believed to be homoxenous, which is responsible for human isosporiasis, a typically cosmopolitan infection, which is more frequent in tropical and subtropical regions. *C. belli* infection is reasonably attributed to the ingestion of the sporulated oocysts in water or contaminated food. All the endogenous reproduction of the parasite occurs in the epithelial cells of the small intestine (Lindsay et al., 1997). In several immunocompromised patients, a unizoic cyst has been observed in the lymph nodes, the spleen, the liver, and the lamina propria of the small intestine (Frenkel et al., 2003a,b; Michiels et al., 1994; Restrepo et al., 1987).

Isosporiasis is characterized by diarrhea, steatorrhea, accompanied by abdominal pains, fever and loss of weight which may lead to dehydration and cachexia. The symptoms are more severe in children with immunity disorders, principally the acquired immuno-deficiency syndrome (AIDS). The rapid dissemination of the HIV virus gave rise to a considerable incidence of this intestinal pathogen, which was considered until the seventies (pre AIDS) to be a parasite of low frequency. (Lindsay et al., 1997).

Although suppressed by sulfamethoxazole–trimetropim therapy, the infection is difficult to eradicate. Recurrences are common and the chronic nature of the illness

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contributes to morbidity and mortality among these patients. (Frenkel et al., 2003a,b).

Several *Isospora* species sensu latu have been studied in cell cultures, with evidence of penetration of the sporozoites into host cells and formation of meronts by endodyogeny (Fayer, 1972; Fayer and Mahrt, 1972; Fayer and Thompson, 1974; Guitiérrez and Arcay, 1987; Lindsay and Blagburn, 1987; Lindsay and Current, 1984), however, there were no data related to the development of *C. belli* in culture cells. In this study the ability of *C. belli* sporozoites to penetrate and multiply as merozoites in different types of mammalian cells was evaluated.

2. Materials and methods

2.1. Parasites

Cystoisospora belli oocysts were isolated from samples of feces of HIV/AIDS patients, which were attending the School Hospital of Universidade Federal doTriângulo Mineiro (UFTM), Uberaba, Minas Gerais, Brazil. The oocysts were sporulated in 2.5% potassium dichromate ($K_2Cr_2O_7$) at room temperature and purified according to the technique of Ortega-Mora et al., 1992; with modifications by Silva et al., 2006: the $K_2Cr_2O_7$ was removed with a phosphate-buffer saline (PBS pH 7.2) containing Tween 20 to 2% (PBS–T20) in 1500 g for 10 min at 4 °C; the lipid portion was removed by means of washing in PBS–T20/ethyilether (2:1) at 1500 g for 10 min; the sediment, which contained oocysts, was added above a non-continuous gradient purification of the 1.05 g/ml and 1.15 g/ml density sucrose and centrifuged at 1500*g* for 20 min at 4 °C; the oocyst layer bas identified between the 2 sucrose layer and was eliminated with 3 washings in PBS at 1500 g for 10 min. After purification, the oocysts were cleaned with 1% sodium hypochlorite solution during 10 min at 4 °C, washed in saline at 1500 g three times and counted a Neubauer chamber. Subsequently, the oocysts were diluted in a phosphate-buffer saline (PBS pH 7.2), containing sodium taurocholate 1.5%, and trypsin 0.5% and incubated at 37 °C for 30 min for excystation (Gold et al., 2001). The sporozoites obtained were diluted in Eagle's minimal essential medium (MEM) supplemented 10% fetal bovine serum (FBS), to neutralize the trypsin, washed for 10 min at 1500 g and counted in the Neubauer chamber. The viability of the sporozoites was evaluated by means of their circular and sporadic movement.

2.2. Cell lines

VERO cells (African green monkey kidney), MDBK (Madin-Darby Bovine Kidney), HCT-8 (Human Ileocecal Adenocarcinoma), and A-549 (Human Carcinoma Lung) were obtained from the ATCC (American Type Culture Collection) and maintained by successive passages in MEM (Gibco-BRL) culture medium, supplemented with 2 mM glutamine, penicillin(10,000 UI/ml)/streptomycin (10,000 μm/ml) solution (Sigma–Aldrich), 0.1 mM non-essential amino acids, amphotericin B (400 μl), and fetal bovine serum (FBS) at 10%. The incubation system used was an open one, with maintenance of the cells in an atmosphere containing 5% of CO_2 at 37 °C, in 12.5 cm^2 culture flasks.

2.3. "In vitro" infection

The cell lines used in the experiment were treated with a solution of trypsin/EDTA 0,05%, neutralized with

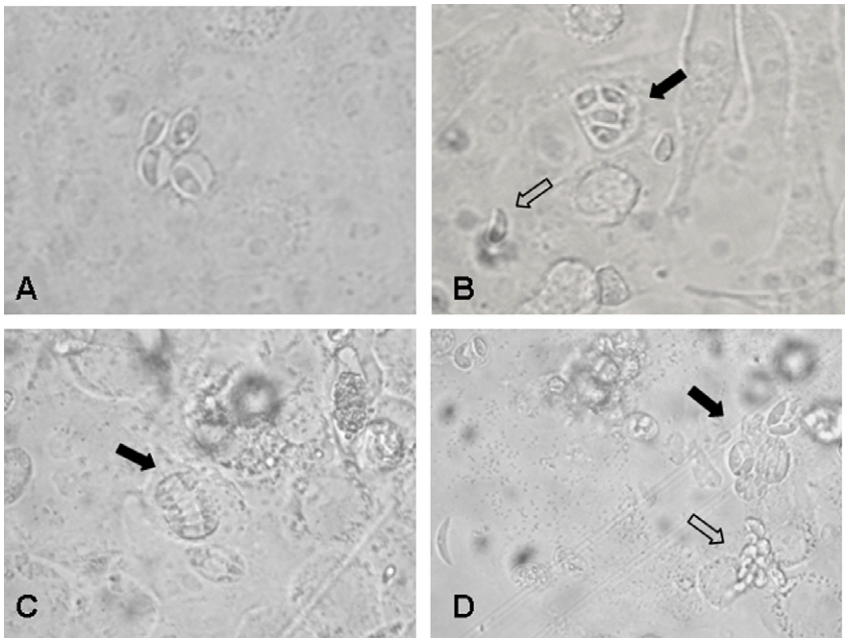

Fig. 1. Fresh culture of VERO and HCT-8 cells infected by *C. belli*: (A) VERO cells with sporozoites in vacuoles 12 h after infection and (B) immature meront 48 h after infection (full arrow) and free merozoite (empty arrow). (C) HCT-8 cells demonstrating division of merozoites in vacuole (full arrow) and (D) immature meronts (full arrow) and escape of merozoites (empty arrow) 72 h after infection. 400×.