

Use of Percoll for the infection of cells in vitro with Cryptosporidium parvum oocysts

Philippe Lawton, Roselyne Mancassola, Muriel Naciri, Anne-Françoise Pétavy

▶ **To cite this version:**

Philippe Lawton, Roselyne Mancassola, Muriel Naciri, Anne-Françoise Pétavy. Use of Percoll for the infection of cells in vitro with Cryptosporidium parvum oocysts. Journal of Microbiological Methods, 2001, 46 (1), pp.81-84. 10.1016/S0167-7012(01)00265-2 . hal-02110972

HAL Id: hal-02110972

https://univ-lyon1.hal.science/hal-02110972

Submitted on 26 Apr 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

ELSEVIER

Journal of Microbiological Methods 1488 (2001) xxx

Journal of Microbiological Methods

www.elsevier.com/locate/jmicmeth

Short communication

Use of Percoll for the infection of cells in vitro with *Cryptosporidium parvum* oocysts

Philippe Lawton [a,*], Roselyne Mancassola [b], Muriel Naciri [b], Anne-Françoise Pétavy [a]

[a] *Département Pharmaceutique de Parasitologie et Mycologie Médicale, Faculté de Pharmacie-8, Université Claude-Bernard, avenue Rockefeller, F-69373 Lyon Cedex 08, France*

[b] *INRA, Laboratoire de Pathologie Aviaire et Parasitologie, Nouzilly, France*

Received 18 December 2000; received in revised form 1 March 2001; accepted 2 April 2001

Abstract

A method for the infection of non-adherent THP-1 cells and adherent MDBK cells with *Cryptosporidium parvum* oocysts using isotonic Percoll solutions was developed. Excystation was maximal after 2 h, but toxicity increased with the oocyst/cell ratio and the incubation time. The infection rates did not increase with the oocyst/cell ratio and both cell types were equally parasitized.

Keywords: *Cryptosporidium parvum*; Culture; In vitro; MDBK; Percoll; THP-1

At present, there is still no ideal method available for the in vitro cultivation of the enteric protozoan *Cryptosporidium parvum*. We previously developed a technique using non-adherent monocytic cells (Lawton et al., 1996) that displays many advantages over the use of monolayers. However, the infection of cells involves either direct contact of the cells with oocysts or addition of purified free sporozoites for various periods of time. When *C. parvum* is cultured in adherent monolayers, high numbers of oocysts are used in order to achieve a correct invasion (Upton et al., 1994a,b; Woods et al., 1995; Griffiths et al., 1998; Theodos et al., 1998; You et al., 1998). Our original protocol, which involved mixing oocysts and THP-1 cells in the culture wells for at least 4 h (Lawton et al., 1996), resulted in many oocysts and sporozoites remaining in suspension while the host cells quickly sedimented, unless periodically resuspended. Thus, to optimize infection, we designed a protocol ensuring that both excysting oocysts and host cells were in close contact, thereby reducing the amount of oocysts needed and the invasion time.

The myelomonocytic cell line THP-1 (ECACC 88081201; Sophia Antipolis, France) was continuously maintained as previously described (Lawton et al., 1996). Madin Darby Bovine Kidney cells (MDBK; ATCC 6071, Rockville, MD) were grown as monolayers in 75-cm^2 Falcon culture flasks (Becton Dickinson, Meylan, France) in the same conditions, except that they were detached with trypsin–EDTA solution (Sigma, L'Isle d'Abeau, France) for routine passaging and prior to the experiments. *C. parvum* oocysts were passaged in neonatal (age 24–48 h) male calves (INRA, Nouzilly, France) and

* Corresponding author. Tel.: +33-4-7877-7277; fax: +33-4-7877-7158.

E-mail address: lawton@univ-lyon1.fr (P. Lawton).

0167-7012/01/$ - see front matter

PII: S0167-7012(01)00265-2

UNCORRECTED PROOF

purified as previously described (Lawton et al., 1996) except that the pooled fecal pellet was allowed to sediment twice in 25-ml cold tap water in order to remove larger debris. Sterile isotonic Percoll was prepared by mixing one part of 10 × Hanks balanced salts solution (HBSS; Sigma H-1641) with nine parts Percoll (Sigma P-4937) and stored at 4°C. Solutions of 30% and 60% were made by diluting the isotonic Percoll with RPMI-1640 and growth medium, respectively. The solutions were stored at 4°C and prewarmed at 37°C before use.

1–4 h prior to infection, the cells were collected and pelleted for 4 min at $160 \times g$. They were suspended in 5-ml growth medium and kept in two wells of a 24-well Falcon microplate (Becton-Dickinson; Meylan, France) (mean concentration: $6.9 \pm 2.2 \times 10^6$ ml^{-1}) in the incubator until required. The MDBK cells were periodically resuspended to minimize their adherence to the bottom of the wells.

The oocysts (approximately $15–50 \times 10^6$) were processed, as previously described (Lawton et al., 1996), and were mixed with the cell suspension from one well (oocyst to cell ratio was 1:1–3:1). The suspension was carefully layered on top of 2.5 ml of the 60% Percoll solution and centrifuged for 10 min at $1500 \times g$ in a 15-ml Falcon centrifuge tube. Excystation was allowed to proceed for 1.5–3 h at 37°C in the parasite–cell layer at the interface between the Percoll solution and the medium. This layer was then retrieved with a Pasteur pipette and washed once in RPMI-1640 for 9 min at $600 \times g$. After dilution with the cells from the remaining well, the parasitized cells were layered on top of a 30% Percoll solution and centrifuged at $1500 \times g$ for 10 min. The pellet was subsequently washed once at $600 \times g$ and thrice at $160 \times g$ as above and distributed at an approximate density of 6×10^6 cells ml^{-1} under reduced oxygen in a candle jar for 24 h. When MDBK cells were used, the 30% Percoll step was omitted. After invasion, the parasitized cells were mixed with the remaining cells, washed thrice at $160 \times g$, distributed in six-well microplates fitted with 22 × 22-mm glass coverslips and allowed to settle and adhere.

After 24 h, the percentage of THP-1 cells infected with *C. parvum* was assessed on thin smears, fixed in 100% methanol and stained with 20% Giemsa and 1% Alcian blue, as previously described (Lawton et al., 1996). The parasite growth in the adherent MDBK cells was also evaluated 24 h post-infection, after the removal of the coverslips from the bottom of the wells. When needed, the cell suspension was diluted 1:10 in saline and counted in a Malassez cell. Toxicity was assessed by Trypan blue dye exclusion. The excystation yield was evaluated by counting the remaining unexcysted oocysts after infection of the cells. The Student's *t*-test was used to assess the statistical significance of the results.

The excystation yield depends on the viability of the oocysts and on the duration of incubation at 37°C. According to Robertson et al. (1993), maximum excystation occurs after 4 h, but the maximum sporozoite yield is achieved after 30 min. Upton et al. (1994b) observed a maximum between 90 and 120 min, and Joe et al. (1998) reported that the attachment of sporozoites to Caco-2 cells reached a maximum after 2 h of incubation. Whatever the oocyst/cell ratio, the percentage of excystation increased when the invasion duration was increased from 1.5 to 2 h, then significantly decreased ($p < 0.05$) at 3 h (Fig. 1). This might be due to the acidification of the medium (pH 6.5) and/or close contact with toxic molecules. However, the damage to the THP-1 cells was significantly more important ($p < 0.05$) as the oocyst/cell ratio increased ($6.1 \pm 2.5\%$, $19.2 \pm 5.1\%$ and $39.2 \pm 4.3\%$ dead cells at oocyst/cell ratios of 1:1, 2:1 and 3:1, respectively).

The infection rates at 24 h p.i. were independent from the oocyst/cell ratio, with about 4.5% of the host cells harboring developing parasites. The infection rates of MDBK cells were comparable to those of THP-1 cells (Fig. 2). The initial percentages of infected cells depend on the availability of host cells and on the invasiveness of the sporozoites. Whatever the cells used in our experiments, monocytic or epithelial, the invasion rates were lower than expected. Several authors have reported comparable infection rates (Slifko et al., 1997; Chen and LaRusso, 2000). This may also be compared to experiments with human volunteers (Chappell et al., 1996) where a minimum of 30 oocysts was necessary to initiate an infection. It is thus possible that only a small fraction of sporozoites are able to successfully multiply into the host cells.

This technique provides maximum contact between host cells and parasites on short periods of

UNCORRECTED PROOF

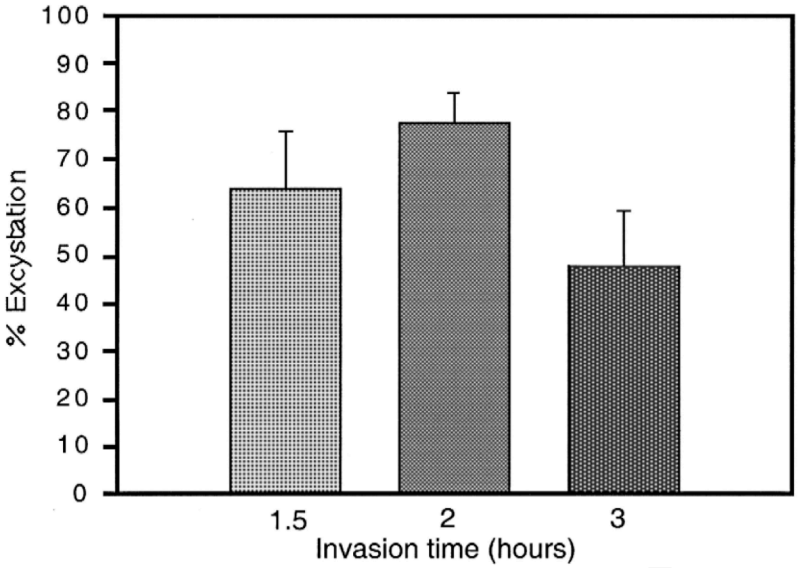


Fig. 1. Efficiency of excystation as a function of incubation time. The maximum excystation yield was achieved after 2 h. THP-1 cells and an 1:1 oocyst/cell ratio were used. Results from three different experiments.

time at an acceptable oocyst/cell ratio. Accordingly, our current standard procedure uses an incubation time of 1.5 h at a 1:1 oocyst/cells ratio. The subsequent use of 30% Percoll also allows the elimination

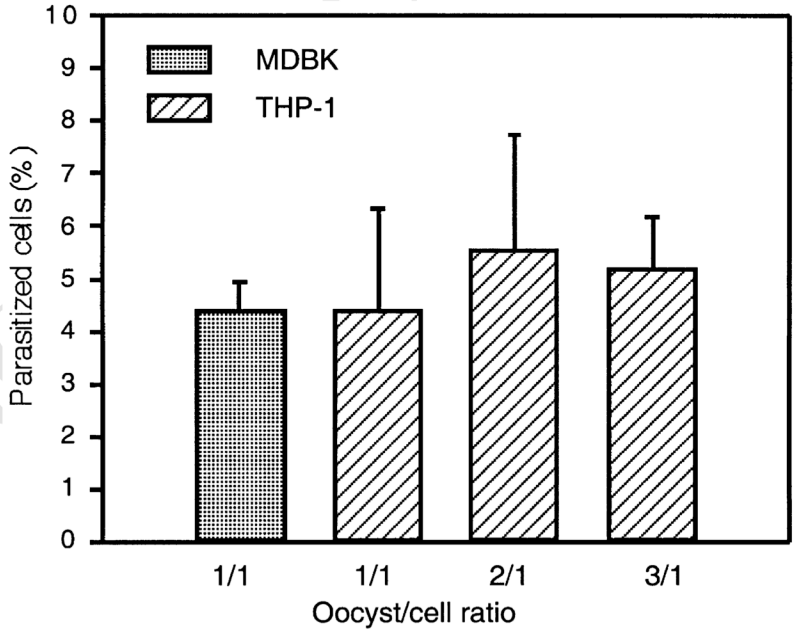


Fig. 2. Percentage of parasitized cells 24 h p.i., with an incubation time of 1.5 h. The mean number of cells harboring developing parasites slightly increased with the amount of oocysts added to the host cells. The infection rates of THP-1 cells and MDBK cells were similar with the same oocyst/cell ratio. Results from three different experiments.

of most of the cell debris and of the oocysts shells, as previously reported (Nesterenko and Upton, 1996) and may also be used in the course of cultivation in order to eliminate dead cells, which can accumulate during these rather stringent culture conditions. However, this step is omitted with MDBK cells because these cells have a wider size heterogeneity, resulting in significant amounts remaining at the surface of the 30% Percoll solution. The present technique designed for non-adherent cells is applicable to epithelial cells and allows the use of the same parasitized adherent cell suspension and its even distribution in culture wells for drug testing.

References

Chappell, C.L., Okhuysen, P.C., Sterling, C.R., Dupont, H.L., 1996. *Cryptosporidium parvum*: intensity of infection and oocyst excretion patterns in healthy volunteers. J. Infect. Dis. 173, 232–236.

Chen, X.M., LaRusso, N.F., 2000. Mechanisms of attachment and internalization of *Cryptosporidium parvum* to biliary and intestinal epithelial cells. Gastroenterology 118, 368–379.

Griffiths, J.K., Balakrishnan, R., Widmer, G., Tzipori, S., 1998. Paromomycin and geneticin inhibit intracellular *Cryptosporidium parvum* without trafficking through the host cell cytoplasm: implications for drug delivery. Infect. Immun. 66, 3874–3883.

Joe, A., Verdon, R., Tzipori, S., Keusch, G.T., Ward, H., 1998. Attachment of *Cryptosporidium parvum* sporozoites to human intestinal epithelial cells. Infect. Immun. 66, 3429–3432.

Lawton, Ph., Naciri, M., Mancassola, R., Pétavy, A.-F., 1996. Cultivation of *Cryptosporidium parvum* in a non-adherent human monocytic cell line. J. Microbiol. Methods 27, 165–173.

Nesterenko, M.V., Upton, S.J., 1996. A rapid microcentrifuge procedure for purification of *Cryptosporidium* sporozoites. J. Microbiol. Methods 25, 87–89.

Robertson, L.J., Campbell, A.T., Smith, H.V., 1993. In vitro excystation of *Cryptosporidium parvum*. Parasitology 106, 13–19.

Slifko, T.R., Friedman, D., Rose, J.B., Jakubowski, W., 1997. An in vitro method for detecting infectious *Cryptosporidium* oocysts with cell culture. Appl. Environ. Microbiol. 63, 3669–3675.

Theodos, C.M., Griffiths, J.K., D'Onfro, J., Fairfield, A., Tzipori, S., 1998. Efficacy of nitazoxanide against *Cryptosporidium parvum* in cell culture and in animal models. Antimicrob. Agents Chemother. 42, 1959–1965.

Upton, S.J., Tilley, M., Brillhart, D.B., 1994a. Comparative development of *Cryptosporidium parvum* (Apicomplexa) in 11 continuous host cell lines. FEMS Microbiol. Lett. 118, 233–236.

Upton, S.J., Tilley, M., Nesterenko, M.V., Brillhart, D.B., 1994b. A simple and reliable method of producing in vitro infections of *Cryptosporidium parvum* (Apicomplexa). FEMS Microbiol. Lett. 118, 45–50.

Woods, K.M., Nesterenko, M.V., Upton, S.J., 1995. Development of a microtitre ELISA to quantify development of *Cryptosporidium parvum* in vitro. FEMS Microbiol. Lett. 128, 89–94.

You, X., Schinazi, R.F., Arrowood, M.J., Lejkowski, M., Juodawlkis, A.S., Mead, J.R., 1998. In-vitro activities of paromomycin and lasalocid evaluated in combination against *Cryptosporidium parvum*. J. Antimicrob. Chemother. 41, 293–296.

UNCORRECTED PROOF