RESEARCH COMMUNICATION

In vitro infection by *Ehrlichia ruminantium* of baby hamster kidney (BHK), Chinese hamster ovary (CHO-K1) and Madin Darby bovine kidney (MDBK) cells

E. ZWEYGARTH and A.I. JOSEMANS

Parasitology Division, Onderstepoort Veterinary Institute
Private Bag X05, Onderstepoort, 0110 South Africa

ABSTRACT

ZWEYGARTH, E. & JOSEMANS, A.I. 2003. *In vitro* infection by *Ehrlichia ruminantium* of baby hamster kidney (BHK), Chinese hamster ovary (CHO-K1) and Madin Darby bovine kidney (MDBK) cells. *Onderstepoort Journal of Veterinary Research*, 70:165–168

The Welgevonden stock of *Ehrlichia ruminantium*, aetiological agent of heartwater, was propagated in baby hamster kidney (BHK) cells, Chinese hamster ovary (CHO-K1) cells and Madin Darby bovine kidney (MDBK) cells. The cultures required supplementation of the medium with cycloheximide for reliable growth of *E. ruminantium*. Growth of the Welgevonden stock in BHK and CHO-K1 cells could lead to the development of suspension cultures suitable for the mass production of *E. ruminantium* for an inactivated elementary body vaccine.

Keywords: BHK cells, CHO-K1 cells, cycloheximide, *Ehrlichia* (*Cowdria*) *ruminantium*, heartwater, *in vitro* cultivation, MDBK cells

INTRODUCTION

Heartwater is a tick-borne rickettsial disease of ruminants caused by *Ehrlichia ruminantium*, the multiplication of which was originally reported to occur within the endothelial cells of infected animals (Cowdry 1926). As a logical consequence of this observation endothelial cells were used for the first successful *in vitro* propagation of *E. ruminantium* (Bezuidenhout, Paterson & Barnard 1985). Subsequent attempts to propagate *E. ruminantium* continuously were therefore carried out almost exclusively in cultures of endothelial cells derived from various animal species and humans. It has been shown that other cells could be infected *in vitro*, including leukocytes (Logan, Whyard, Quintero & Mebus 1987) and monocytes (Sahu 1986), but not fibroblasts (Bezuidenhout 1987). Nevertheless, Da Graça (1966) described *E. ruminantium* in fibroblasts of the interstitial spaces and alveolar septa of the lung of a ewe, and Ilemobade (1976) described the presence of *E. ruminantium* in the epithelium of a renal tubule of one experimentally infected ox. It was recently shown that the Kümm isolate (Du Plessis & Kümm 1971) consisted of two distinct genotypes, both of which were isolated and propagated in cells of non-endothelial origin (Zweygarth, Josemans, Van Strijp, Van Heerden, Allsopp & Allsopp 2002). These observations prompted us to further investigate the possibility that *E. ruminantium* could enter, survive and grow in cells of non-endothelial origin. We used baby hamster kidney

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TABLE 1 Propagation of the Welgevonden stock of *E. ruminantium* in the BHK, CHO-K1 and MDBK cell lines

Host cells	Number of passages	Passage intervals [days]
Baby Hamster Kidney cells (BHK)	33	3
Chinese Hamster Ovary cells (CHO-K1)	26	3
Madin Darby Bovine Kidney cells (MDBK)	11	3

(BHK) cells, morphologically described as being fibroblast-like, and Chinese hamster ovary (CHO-K1) and Madin Darby bovine kidney (MDBK) cells, morphologically described as being epithelial-like.

MATERIALS AND METHODS

Stock of *E. ruminantium*

The Welgevonden stock of *E. ruminantium* was used for the experiments (Du Plessis 1985).

Cells and cell lines

Four different cell lines were used, one of which was endothelial. A bovine aorta cell line (BA 886) (Yunker, Byrom & Semu 1988) was used for the initial propagation of the Welgevonden stock of *E. ruminantium*. BHK cells and MDBK cells were obtained from the Division's cryobank. CHO-K1 was purchased from Highveld Biologicals, Kelvin, South Africa. The passage history of these cell lines is unknown.

Infected and uninfected cells were propagated at 37 °C in a medium consisting of Dulbecco's modified Eagle's medium nutrient mixture F-12 Ham (Sigma, St. Louis, MO, USA) with 10 % (v/v) heat-inactivated foetal bovine serum (FBS; Delta Bioproducts, Kempton Park, South Africa). The medium was buffered with 15 mM HEPES (N [2-hydroxyethyl]piperazine-N'-[2 ethanesulfonic acid]) (Sigma) and 1.2 g/ℓ sodium bicarbonate, and supplemented with 2 mM L-glutamine, 100 IU/mℓ penicillin and 100 µg/mℓ streptomycin.

Propagation of cultures

Endothelial cell cultures heavily infected with *E. ruminantium* were harvested by scraping the cell monolayer off the walls of the culture flask into the medium. The cell suspension was centrifuged (800 x *g* for 10 min at room temperature) after which the supernatant contained predominantly elementary bodies. Varying amounts of supernatant, up to 2.5 mℓ, were distributed into culture flasks containing the host cells. Attempts were made to initiate two different types of culture, one using the medium described above and the other in which the same medium was supplemented with 0.5 µg $m\ell^{-1}$ or 1 µg $m\ell^{-1}$ cycloheximide (CyX). The cultures were incubated at 37 °C and the medium was removed after 24 h and replaced with 5 mℓ of the respective medium. When cellular infections had been initiated subsequent passages were carried out using infected cell suspensions which were not centrifuged beforehand.

Microscopic examinations were carried out to demonstrate the presence of *E. ruminantium* organisms in the host cells. Small cell samples were removed by means of a sterile 21-gauge needle with a bent tip, and smears were prepared. They were air-dried, methanol-fixed, and quick-stained (Rapi-Diff; Clinical Sciences Diagnostics, Booysens, South Africa).

RESULTS

Three days after inoculation with elementary bodies obtained from bovine endothelial cell cultures it was observed that all cell lines were infected and contained colonies. The first culture passage was carried out on day 3. The addition of CyX to the culture medium was necessary for continuous cultivation and to achieve regular passage intervals. Infected BHK cultures were kept for 33 passages, infected CHO-K1 cultures for 26 passages, whereas infected MDBK cultures were terminated after 11 passages. These results are summarized in Table 1.

DISCUSSION

Continuous *in vitro* propagation of *E. ruminantium* has been restricted to endothelial cell cultures until recently. Early unsuccessful attempts to propagate *E. ruminantium* in fibroblastoid cell lines include the inability of Vero cells and lamb foetal kidney cells to support the growth of the Ball 3 isolate, and of mouse L-cells to become infected with the Welge-

vonden stock (Bezuidenhout 1987). The situation changed, however, when DH82 cells were infected with the Welgevonden stock of *E. ruminantium* and propagated continuously for more than 100 passages (Zweygarth & Josemans 2001a). It has also been reported that the Kümm isolate of *E. ruminantium* was propagated in a cell line of non-endothelial origin (Zweygarth *et al.* 2002)

In the present experiments we demonstrated that *E. ruminantium* can enter, survive and grow in cells of non-endothelial origin, morphologically described as being fibroblast-like (BHK) or epithelial-like (CHO-K1, MDBK). However, all infected cell lines required the presence of CyX for a regular growth pattern with regular subculture periods and consistent subculture ratios. Cycloheximide is a specific inhibitor of protein synthesis in eucaryotic cells (Ennis & Lubin 1964) which has been used to promote the growth of chlamydial organisms in cell cultures (Hobson, Johnson & Byng 1977), and also of *E. ruminantium* in DH82 cells (Zweygarth & Josemans 2001a). The propagation of the cultures without CyX was abandoned because of irregular growth patterns (data not shown) although it cannot be excluded that they could have been potentially viable.

The culture of *E. ruminantium* in non-endothelial cells, as described here, could have a practical implication for the bulk production of *E. ruminantium* elementary bodies for use in an inactivated elementary body vaccine (Martinez, Maillard, Coisne, Sheikboudou & Bensaid 1994; Mahan, Andrew, Tebele, Burridge & Barbet 1995). A three-step protocol for adapting an anchorage-dependant, serum-dependant, cell lineage of recombinant CHO cells to a serum-free suspension culture has been described (Sinacore, Drapeau & Adamson 2000), and media for the serum-free propagation of *E. ruminantium* have also been developed (Zweygarth, Vogel, Josemans & Horn 1997; Zweygarth, Josemans & Horn 1998; Zweygarth & Josemans 2001b). Our present results therefore suggest that a large-scale production system for *E. ruminantium* could be feasible, using either CHO-K1 or BHK suspension cultures as the host cells. Such a system would be more convenient than the present one, in which collagen microspheres as carriers for endothelial cells are used (Totté, Blankaert, Marique, Kirkpatrick, Van Vooren & Wérenne 1993). While the transition from *E. ruminantium*-infected stationary cultures to suspension cultures is likely to present some difficulties, there is a real need for a large scale, economical production system for this organism.

In conclusion, it has been shown that the Welgevonden stock of *E. ruminantium* was able to grow in non-endothelial cells from different animal species, both ruminant and non-ruminant. Whether *E. ruminantium* infects non-endothelial cells other than leukocytes and monocytes/macrophages *in vivo*, however, remains to be elucidated.

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