

Toxoplasma gondii in vitro cultivation: easy handling long-term propagation

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Abstract

Based on a serum-free and CO₂-independent culture of *Toxoplasma gondii* in vitro, a long-term propagation of a highly virulent strain of this parasite was developed. *Toxoplasma* monolayer cultures were fed on a weekly cycle with native host cells (HEp-2) while changing the medium, thus simultaneously removing cell debris and just bred trophozoites. Establishing this method was of low effort in organisation and alterations, as the technique is harmonised with a routine laboratory activity. We were successful in propagating the parasite in vitro for half a year without any loss of infectivity or decrease of the rate of multiplication. The advantages of this in vitro model are a considerable reduction of the financial expenditure on one hand and of the risk of a contamination on the other.

Keywords: Cultivation in vitro; HEp-2 cells; Long term propagation; *Toxoplasma gondii*

1. Introduction

Usually the obligatory parasite *Toxoplasma gondii* is propagated either in the peritoneal cavities of mice (in vivo) or in tissue cultures (in vitro). In vitro cultivation using secondary mammalian cells as hosts is animal protective but much more complicated and labour-extensive than in vivo culture. There is a number of instructions for different types of *Toxoplasma* cultures in vitro, but most of these techniques require a fumigation chamber, an obligatory addition of expensive nutrients, and high manual labour input, especially if suspension cultures are performed [1].

The development of a serum-free and carbon

dioxide (CO₂)-independent in vitro culture of *Toxoplasma gondii* [2,3] allows the application of an easy handling procedure with a 15% cost reduction for equipment goods and about 30% decrease of the current expenses [2]. The basis of this technique is a monolayer tissue culture using HEp-2 cells as hosts and rapidly dividing trophozoites of the virulent *Toxoplasma* BK strain [2].

Usually there are two basic reasons for a parasite cultivation in vitro. First, a long time continuing preservation of the strain without resorting to laboratory mammals, and secondly, a distinct, cheap production of large numbers of parasite cells, especially for an application as antigen. But, it seems to be infeasible to unite both aims within one cultivation strategy, at least within our procedure. Therefore we developed two distinct procedures for a long-term

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propagation on one hand and a mass production on the other hand, both based on the serum-free and CO₂-independent tissue culture.

2. Materials and methods

For the tissue cultures, e.g. culture of uninfected host cells on one hand and of parasites on the other, a mixed nutritive medium was used, containing one part of the PC-1 medium (Hycor, Irvine, CA, USA) and one part of the CO₂-independent medium (Gibco, Gaithersburgh, MD, USA). This mix was enriched with 1% L-glutamine (Life Technologies Ltd, Paisley, GB), 1% antibiotics/antimycotics (Life Technologies), and 1% serum supplement (Hycor).

As hosts HEp-2 cells were used (CCI 23, American Type Culture Collections (Rockville, MD, USA)), the parasite strain cultivated was the BK strain from Vienna (strain characteristics: [4]). The cultivation took place in 75 cm² flasks (Costar, Cambridge, MA, USA), these flasks require 15 ml medium for optimal running. Medium handling was done with 10 ml plastic pipettes (Falcon; Becton Dickinson, Franklin Lakes, NJ, USA), and cells were bred at 37°C without any fumigation or agitation.

2.1. Host cell cultivation

A total of 10⁶ HEp-2 cells were incubated for 24 h, then the medium was changed, thus removing non-attached cells at the same time, and after a further 24 h a mean of 1.5 × 10⁷ cells (SD: ±0.3) could be harvested using 1 ml 0.25% trypsin (Life Technologies) for detachment. The cells were counted in a Bürker-Türk counting chamber.

2.2. Parasite cell cultivation

A new culture was started by mixing 10⁶ HEp-2 cells and 10⁶ parasites bred in vivo in 15 ml nutrition medium. After a 24 h incubation period the first medium change was performed, and after another 24 h an endless weekly cycle (Fig. 1) could be applied. One cycle consisted of time periods of 48 h, 48 h, and 72 h between the handling of feeding and harvesting.

The supernatant of the cultures was aspirated,

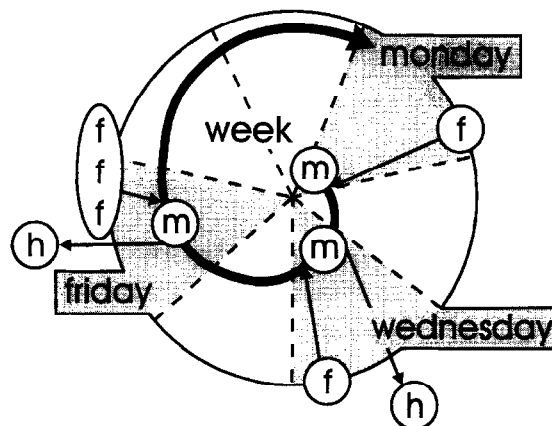


Fig. 1. Schema of the weekly cycle of a permanent *Toxoplasma* strain propagation. m, exchange of the nutrition medium; f, feeding of the parasites with 10⁶ native host cells; h, harvesting extracellular trophozoites (about 2.7 × 10⁷).

thereby removing all extracellular parasites which can be used for starting the mass production (see Part 2). Fifteen ml of fresh medium were added to the remaining monolayer, containing 10⁶ native HEp-2 cells. Every second day the feeding was repeated, except for each third cycle, where 3 × 10⁶ host cells were added and the incubation period was extended to 3 days — for spanning the weekend, or a public holiday.

3. Results and discussion

This technique was tested in five parallel attempts and each was successfully run for 6 months, until a deliberated termination. During that time a mean of 1.5 × 10⁹ (SD: ±0.5) trophozoites were harvested from one culture flask, requiring an input of 1.3 × 10⁸ HEp-2 cells and 1.22 l nutrition medium. Moreover, we have cultivated eight different virulent *Toxoplasma* strains for a long time [2], but a cultivation for six months was performed with BK-Vienna only.

The main problem of any continuous *Toxoplasma* culture in vitro is the permanent loss of infectivity of the trophozoites. If newly bred extracellular parasites are unable to invade native host cells within a few seconds, their mobility decreases rapidly. Thus, their chance in finding an attached host cell decreases — until their complete exhaustion. So, within an in vitro

bred parasite population, the rate of non-infective, extracellular trophozoites increases constantly [5]. Thus, by regularly removing the extracellular trophozoites during medium exchange only newly emerging, highly infective parasites are left. These parasites can easily find new, native host cells, as such cells are added to the culture (feeding). By this procedure the period of extracellular stay of the parasites is shortened as much as possible, thus, the dreadful loss of infectivity is completely prevented. The number of host cells to add during a feeding step depends on the culture technique used and on their multiplication and depletion characteristics. We decided to base our propagation procedure on our well-defined HEp-2 culture model, as the aim was the development of a stable, easily manageable technique for a long-term cultivation in a routine laboratory.

Other authors, too, have tried to reduce the time of the extracellular stay of the parasites [6–9]. They artificially disintegrated infected host cells by mechanical disruption. However, this method is sophisticated, tissue damaging, and labour intensive and cannot be integrated into a routine culture cycle.

Nevertheless, the trophozoites harvested are contaminated with cells and cell remnants to a large extent (10–15% w/w). So, beside the relative small number of parasites harvested, they are unsuitable for protein studies or for an antigen extraction. Mass production of almost pure parasites must be done using a different technique. The method described convinces by its low labour expenditure, low organisation amount, and by a very low contamination risk, thus being perfectly suited as a routine procedure.

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