IN VITRO PROPAGATION OF TOXOPLASMA GONDII IN PC-1 SERUM-FREE MEDIUM

So far, in vitro cultivation of Toxoplasma gondii (Nicolle et Manceaux, 1908) has always been performed in serum or yeast-extract enriched media, thereby introducing an uncontrolled set of proteins into the parasites environment. Moreover, serum containing propagation techniques always include the risk of introducing (diaplastically transmitted) anti-Toxoplasma antibodies. Until now, one notice has only been published describing the cultivation of T. gondii in host cells raised in serum-free media (SHITE and Iscove’s medium) (Hermentin et al. 1987; J. Parasitol. 73: 1276–1277). However, these media had to be enriched with growth-stimulating supplements, making serum-free Toxoplasma cultivation complicated and expensive. Thus, we searched for an easy-to-use serum-free nutrition medium which would enable the simultaneous multiplication of both host tissues and Toxoplasma parasites. We cultivated the parasites (strain BK, virulent) in HEP-2 host cells (CCI 23 ATCC; 2 × 10⁶ tachyzoites to 5 × 10⁵ host cells in 25 cm² flasks) raised in the ready-to-use, serum-free and low-protein medium Ventrex PC-1 (Ventrex Laboratories Inc; Portland; ME) on one hand (sf culture technique) and in Earle’s medium with 10% heat inactivated fetal calf serum on the other (conventional (conv.) culture technique) according to the technique described by Hermentin et al. (1987; Zbl. Bakter. Hyg. A 267: 272–276). All cultures were harvested on day 4 p.i.; all data of three consecutive experiments were evaluated. For comparison, T. gondii tachyzoites were also routinely maintained in the usual way by continuous intraperitoneal passages in SPF-mice (OF 1-Swiss, n = 20, three experiments) (43 h cycle).

On day 4 p.i. about 8 × 10⁷ tachyzoites were harvested in both tissue cultures, the Toxoplasma tachyzoites had multiplied about 40-fold (conv: x = 42, SD 2.5; sf: x = 40, SD 1.76; statistically NS, t-test). The multiplication rate of the parasites in the mouse peritoneal cavity was about 15-fold within 43 hours. Although there is almost the same quantity of parasites maturing in both types of tissue cultures, there is a statistically highly significant (n > 90; p < 0.0005; Welch-test) difference in the parasite size (length: conv: x = 4.01 μm, SD 0.44; sf: x = 5.74 μm, SD 1.0; mouse: x = 5.0 μm, SD 0.71); size measurement done with an ocular micrometer. The tachyzoites increases in the average size by about 40% in PC-1 medium, they even become more voluminous than those grown in the mouse peritoneal cavity. It seems that the parasite “feels quite comfortable” during its multiplication in the PC-1 medium poor of any inhibiting factors.

As Toxoplasma tachyzoites seem to adsorb some environmental proteins, especially antibodies, to their surface (e.g. Budzko et al. 1989: J. Clin. Microbiol. 27: 959–961) the antigen composition of the surface may change considerably during any serum-free propagation. Thus, we compared the major proteins of T. gondii in a reducing SDS-PAGE (2 × 10⁵/ml tachyzoites, gradient gel 10–15, PhastSystem; Pharmacia LKB Biotechnology, Vienna; staining with Coomassie blue; all done according to the manufacturer’s recommendations). As can be seen in the image (Fig. 1) from the gel, scanned in a PhastImage and redrawn with a graphic software, protein composition of the parasites grown under different environmental conditions do not change considerably, except in the low molecular weight area (mw < 30 kD). Yet, this coincidence might be due to differences in the protein shattering during preparation. Note worthy, the peak with a molecular weight of about 30 kD in conventional tissue culture shifts; just this protein is a very prominent Toxoplasma surface antigen (e.g., Kasper et al. 1983: J. Immunol. 130: 2407–2412). Thus, before using this in vitro propagation technique for an antigen production for sero tests the diagnostic relevance of such serological assays has to be verified.

So far, this is the first report of a propagation of T. gondii in Ventrex PC-1 medium, a serum-free, low-protein tissue culture medium, which is – according to the producer – a modified DME/F12 base with less than 530 μg proteins/ml (further details are not available). No adaption either of
Fig. 1. Graph of a dyed and scanned polyacrylamid-gel after a SDS-electrophoreses under reducing conditions. The samples were lytic proteins of *Toxoplasma gondii* trophozoites grown in mouse peritoneal cavity (dotted line), in a serum enriched HEP-2 tissue culture (dashed line), or in a PC-1 serum-free HEP-2 tissue culture (unbroken line). The dotted line was 50% upset and lifted. From both graphs of tissue culture grown *Toxoplasma* (dashed and unbroken line) all peaks were eliminated which were identical with peaks prepared from uninfected HEP-2 cells. The triangles mark the sites of commercially available molecular weight markers (from left: 94, 67, 43, 30, 20, 1, and 14.4 kD).

The HEP-2 cells or of the parasites to the serum-free environment by a gradual decrease of serum concentration in the nutrition medium has been necessary for their propagation. Thus, a very convenient and reproducible technique for an *in vitro* multiplication of *T. gondii* is offered, which produces rich yields of remarkably large parasites.

This study has in part been supported by the grant P7151-MED of the Austrian Fonds zur Förderung der wissenschaftlichen Forschung.

A. HASSL and H. ASPÖCK

Department of Medical Parasitology,
Clinical Institute of Hygiene,
University of Vienna,
Kinderspitalgasse 15, A-1095 Vienna, Austria.

Received 30 December 1991

Accepted 2 March 1992