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A Rapid and Simple Method of Purification of Toxoplasma gondii Trophozoites Originating from Tissue Culture for Use in the Indirect Immunofluorescent Antibody Test*

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Abstract

A simple and convenient purification method for *Toxoplasma* trophozoites from tissue culture cells by density centrifugation is described. Separation of cells treated with formaldehyde is achieved by means of a Percoll solution with a density of 1.056 g/ml permitting removal of more than 99% of the cells and cell debris from the tissue culture. Thus highly purified trophozoites of *Toxoplasma gondii* raised in tissue cultures become available for serological tests, particularly for the indirect immunofluorescent antibody test.

Zusammenfassung

Eine einfache und praktische Methode zur Reinigung von Toxoplasma-Trophozoiten von Zellkulturrückständen mittels Dichtezentrifugation wird beschrieben. Die Trennung der Formaldehyd-fixierten *Toxoplasma*- von den Wirtszellen wird durch eine Percoll-Lösung der Dichte 1.056 g/ml durchgeführt, wodurch über 99% der Wirtszellen entfernt werden. Dadurch können Toxoplasma-Trophozoiten aus der Gewebekultur in hohem Reinheitsgrad für den Indirekten Immunfluoreszenztest gewonnen werden.

Introduction

The use of tissue cultures for the multiplication of *Toxoplasma gondii* is constantly gaining importance due to aggravating animal welfare regulations. However, unlike *Toxoplasma* trophozoites obtained from the mouse peritoneal cavity, *Toxoplasma* suspensions produced in tissue cultures are heavily contaminated with host cells and

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cell debris (5). In some serotests, especially in the indirect immunofluorescent antibody test (IFAT), which is the basis test for toxoplasmosis screening during pregnancy in Austria (1), this contamination is a highly disturbing factor. Therefore, a purification method for the IFAT-antigen is needed which should be simple, rapid, and convenient for routine processing.

Material and Method

Toxoplasma gondii, strain BK, was maintained in HeLa, Vero, and HEp-2 cells according to the optimal procedure recommended by *Hermentin* et al. (4). Four days after infection, the *Toxoplasma* trophozoites were harvested and processed into IFAT antigen roughly following the recommendations of the Bundesgesundheitsamt of the FRG (2). The trophozoites and the contaminating host cells were counted in a Bürker chamber and then pelleted at 25 g (5 min). After having been washed twice in 0.15 M NaCI + heparin (25 I.U./ ml; Immuno AG, Vienna, Austria) (25g, 5 min), they were resuspended and incubated in 0.15 M NaC1 + 0.4% formaldehyde + heparin (25 I.U./ml) for 10 min. After a third washing step, the trophozoites and cells were ready for separation.

First, it had to be ascertained whether *Toxoplasma* trophozoites and host cells (HEp-2) had different densities. Therefore an iso-osmotic Percoll gradient (Pharmacia Ges.m.b.H., Vienna) with a density between 1.025 and 1.11 g/ml was established, and by centrifugation at 400 g for 15 min, the cells were distributed following this gradient according to their densities. Then, the density gradient was carefully divided into fractions with a volume of one ml each, and the densities of the single bands were calculated.

In a further step, the optimal density of the Percoll solution used for cell separation in a single-dilution gradient was determined. Trophozoites and 'host cells were counted in a Bürker chamber, and 1 ml of this suspension was layered on top of the Percoll solutions of different densities (30-70% iso-osmotic Percoll in 0.15 M saline). After centrifugation, the pellet was resuspended in 1 ml saline and the pelleted cells were counted.

As a last step, the optimal procedure was repeated several times with the host cells in different conditions of destruction, with Vero cells and Vero-derived *Toxoplasma,* with HeLa cells and HeLa-derived *Toxoplasma,* with Tween 20 instead of heparin, and, finally, the maximum sample load was determined. The antigenicity of the IFAT antigen prepared as described was compared with that of antigen originating from the mouse peritoneal cavity by comparative testing of 30 human sera with different IFAT titres (negative to 1 : 256). The IFAT was performed according to the recommendations of the Bundesgesundheitsamt (2).

Results

Toxoplasma trophozoites could be separated from host cells by density centrifugation. The densities of the different cell types are listed in Table 1. In Table 2 the results of purification of *Toxoplasma* trophozoites by density centrifugation with different dilutions of stock Percoll solution are presented. The best results were obtained with a 45% iso-osmotic Percoll solution (density: about 1.055 g/ml). As the number of host cells remained less than 0.1% of all particles, far more than 99% of the host cells had been removed. No significant differences between the densities of *Toxoplasma* trophozoites originating from HeLa, Vero, or HEp cells could be found. The maximum quantity of *Toxoplasma* trophozoites suitable for centrifugation in our system was about 5x 107 cells in 1 ml saline overlaying 5 ml Percoll solution. The use of Tween 20

Table 1. Densities of cells and *Toxoplasma* trophozoites treated with formaldehyde as determined by density centrifugation with iso-osmotic Percoll diluted in 0.15 M saline. %: Percentage of iso-osmotic Percoll in the final dilution (v/v)

Cell Type	Density (g/ml)	%
HEp-2 cells	1.031-1.037	25-30
Infected HEp-2 cells and host cells stuck with *Toxoplasma* cells	~1.053	~42
Pure *Toxoplasma* cells	1.09-1.11	75-85

Table 2. Recovery of *Toxoplasma* trophozoites and HEp-2 cells, and percentage of HEp-2 cells within all counted particles (scale of impurity) (%) after density centrifugation with different dilutions of iso-osmotic Percoll. Values are means ± standard deviations (both rounded). n.d. = not detectable

Iso-osmotic Percoll in the solution	Number of *Toxoplasma* cells (x 10^5)	Number of HEp-2 cells (x 10^5)	%
source	51.0 ± 12	13.0 ±5.6	25.5
control = 0%	10.3 ± 6.1	2.5 ± 1.7	24.4
30%	4.0±4.0	1.5±2.1	37.5
35%	4.8±3.0	0.8±1.2	16
40%	6.5±5.8	n.d.	<0.1
45%	9.2±6.9	n.d.	<0.1
50%	7.0±4.8	n.d.	<0.1
55%	3.3±3.1	0.8±1.2	23
60%	2.3±1.9	0.3±0.8	11
65%	1.8±1.7	n.d.	-
70%	0.5±1.0	n.d.	-

for spreading is not recommended. When used in the IFAT, there were no significant differences found in the titres obtained with antigen which originated from the mouse peritoneal cavity and antigen which had been prepared as described below.

Discussion

A drawback of *Toxoplasma* multiplication in tissue cultures is the unavoidable contamination of the trophozoites harvested with host cells. Especially in the IFAT, this impurity of the antigen is frequently found to be a serious disadvantage. Although various purification methods for *Toxoplasma* trophozoites have been described, the use of e. g. lectins (6), paper filters (7), glass filters (3), zonal density centrifugation (8),

or disruption of host cells (9), no simple and rapid method suitable for routine processing has been published for the isolation of Toxoplasma trophozoites from uniform tissue culture cells.

The optimal procedure for purification of Toxoplasma-IFAT antigen from tissue culture debris is as follows

Prepare your IFAT antigen as usual;
during the last washing step but one
↓
Resuspend carefully <= 5 x10^7 cells in 1
ml 0.15 M saline + 25 I.U. heparin
↓
Prepare Percoll solution:
2025 µl pure Percoll + 500 t1 1.5 M saline
+ 125 I.U. heparin
Make up to 5 ml with distilled water
↓
Overlay Percoll solution with cell suspension
centrifuge at 400 g for 15 min
↓
Suck off supernatant except bottom 0.5 ml + pellet
↓
Resuspend in 0.15 M saline and wash for the last time

The purification method described is an easy-to-perform, inexpensive, and convenient method for separation of Toxoplasma trophozoites from host cells. As the cells are treated with formaldehyde, no special care must be taken to avoid cell rupture; without a previous formaldehyde treatment, high losses due to extensive cell destruction during the centrifugation steps were observed (unpublished data). The recovery rate of Toxoplasma trophozoites after the whole procedure is about 20% (Table 2); it is far higher than the one obtained by most filtration methods. However, the recovery rate may strongly depend on the ratio between host cells and free trophozoites, due to cell agglutination and encasing. The development of a method for an effective spreading of the cells without any alteration of the characteristics of sedimentation may dramatically increase the recovery rate. Furthermore, the use of the purification method described yields a very homogeneous antigen, as altered or deformed trophozoites are removed which is a considerable advantage for the application in an indirect fluorescent antibody test.

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