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Original Article

Cyst Formation from Virulent RH Strain of *Toxoplasma gondii* Tachyzoite: In Vitro Cultivation

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Abstract

Background: This study was performed to induce conversion of RH strain tachyzoites of *Toxoplasma gondii* to bradyzoites by pH changing of the culture medium.

Methods: HeLa cell monolayers were infected at a 1:1 tachyzoite to cell ratio. Four hours after infection, the culture medium was removed and replaced with culture medium and 5% FCS, adjusted to pH 8 with NaOH. The culture was maintained at 37 °C without CO₂ until the end of the experiment. Cyst-like structures were collected and stained with periodic acid schiff (PAS) staining method. The soluble antigens of cyst-like structures of RH strain, in addition to RH tachyzoite, bradyzoites of avirulent Tehran strain and uninfected HeLa cells were electrophoresed on 12.5% polyacrylamide gel. The gel was stained by coomassie brilliant blue R-250.

Results: Four days after infection of HeLa cells with tachyzoites of *T. gondii*, RH strain, cyst-like structures were noticed and stained with PAS. In the SDS-PAGE, protein bands of these structures had some differences with tachyzoites of RH strain, but there was quite similarity between protein bands of these structures and tissue cysts (bradyzoites) of Tehran strains. P34 and P36 (bradyzoite-specific proteins) were observed only in *T. gondii* bradyzoites of RH (cyst like structures) and bradyzoites of Tehran strains.

Conclusion: Alkalization of culture medium to pH 8 induced expression of bradyzoite-specific proteins and production of RH cysts in cell culture.

Introduction

Toxoplasma gondii is an intracellular protozoan that infects mammals and birds. An infection by the *T. gondii* is widespread and is of economic and public health importance (1, 2). It has a complex life cycle involving both sexual and asexual reproduction. In intermediate hosts the parasite exists in two distinct stages; the proliferative tachyzoite, responsible for the acute phase of the infection, and the bradyzoite which forms persistent tissue cysts in brain and muscles (3, 4). Tissue cysts are able to reconvert into tachyzoites in immunocompromised patients (5). The mechanism of tachyzoite-bradyzoite interconversion is poorly understood.

Immunologic factors are not required for cyst formation, and some *Toxoplasma* strains that produce tissue cyst in mice can spontaneously develop tachyzoite and bradyzoite in cell culture (6, 7).

The present study was performed for conversion of tachyzoites of *T. gondii*, RH strain to bradyzoites by pH changing in cell culture.

Materials and Methods

T. gondii tachyzoites preparation

Tachyzoites of *T. gondii* (RH strain) were inoculated intraperitoneally in BALB/c mice. After 72 h tachyzoites were harvested by intraperitoneal washing with sterile phosphate buffered saline (PBS, pH 7.3).

This study was approved by ethical committee of Tehran University of Medical Sciences and performed in 2014.

HeLa cells culture

HeLa cells obtained from Virology Department, Iran University of Medical Sciences, Tehran, Iran were grown in 25cm² flasks (Nunc, Denmark) in 10 ml of culture medium: Dulbecco's modified eagle medium (DMEM; KBCell, Iran) supplemented with

10% inactivated fetal calf serum (FCS; Bovogen, Australia), 10 mM hepes and 1% penicillin- streptomycin (Biowest, France) and incubated at 37 °C in a 5% CO₂ atmosphere. When a confluent monolayer was obtained, the medium changed to DMEM/ hepes with 5% FCS (maintenance medium). Subculture was done weekly by adding trypsin to confluent monolayers and washing with sterile PBS (pH 7.3).

In vitro culture of *Toxoplasma gondii*

HeLa cell monolayers were infected with tachyzoite at a 1:1 ratio. After 4 h, when active tachyzoites had entered into the cells, the media was removed and replaced with culture medium and 5% FCS, adjusted to pH 8 with NaOH. To avoid pH variation, the culture was maintained at 37 °C incubator without CO₂ until the end of the experiment.

Periodic acid schiff (PAS) staining

After removal of the culture media, infected cells were scratched from the bottom of the flask. Smears of these cells were prepared on microscopic slides and air dried. Then they were fixed in absolute methanol and allowed to dry. Periodic acid 0.5% was added for 5 min, rinsed with water, schiff reagent was placed for 20 min, washed in tap water for 5 min; Mayer's hematoxylin was applied for 1 min, washed in tap water for 5 min. Finally, the slides were mounted and examined by light microscopy with 100X magnification.

SDS-PAGE

The soluble antigens of *T. gondii* tachyzoites and bradyzoites (cyst like structures) of RH strain, bradyzoites of Tehran strain and uninfected HeLa cells (30 µg/ well) were mixed with sample buffer and heated for 2 min at 90 °C respectively. Fifty microliters of each soluble antigen and molecular weight marker (chromatein prestained protein ladder, Vi-

vantis) were run on 12.5% polyacrylamide gel and electrophoresed at 150V for 3 h. The gel was stained by coomassie brilliant blue R-250 (Sigma, USA) over night at room temperature. The protein profile for each sample was characterized.

Results

Four days after infection of HeLa cells with tachyzoites of *T. gondii* RH strain, cyst-like structures were obtained in vitro (Fig. 1).



Fig. 1: Purified cyst-like structure of *Toxoplasma gondii*, RH strain in cell culture with pH adjusted to 8 (without staining), 100X (Original picture)

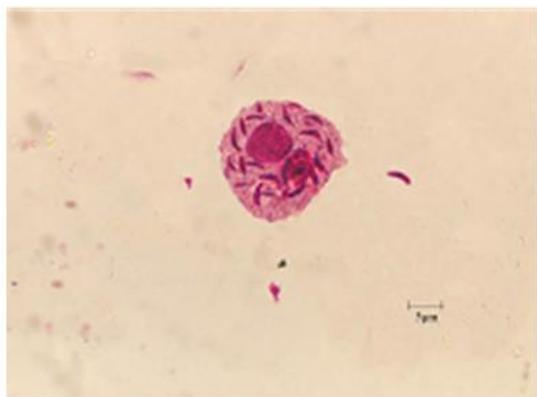


Fig. 2: Cyst-like structure of *Toxoplasma gondii*, RH strain in cell culture with pH adjusted to 8, (PAS staining), 100X (Original picture)

Cyst-like structures were noticed when they stained with PAS method that stains amylopectin granules in bradyzoites. Figure 2 shows the organisms in red color, demonstrating several amylopectin granules in them. Cyst-like structures were about 15µm in size with well-defined walls and contained numbers of bradyzoite. The morphology of these structures was similar to cyst purified from the brain of mice infected with avirulent Tehran strain at light microscopy.

In SDS-PAGE, the protein bands were not quite similar between *T. gondii* tachyzoites and bradyzoites (cyst-like structures) of RH strain but quite similar between *T. gondii* bradyzoites (cyst-like structures) of RH and avirulent Tehran strain. P34 and P36 (bradyzoite-specific proteins) were observed only in *T. gondii* bradyzoites (cyst-like structures) of RH and avirulent Tehran strains (Fig. 3).

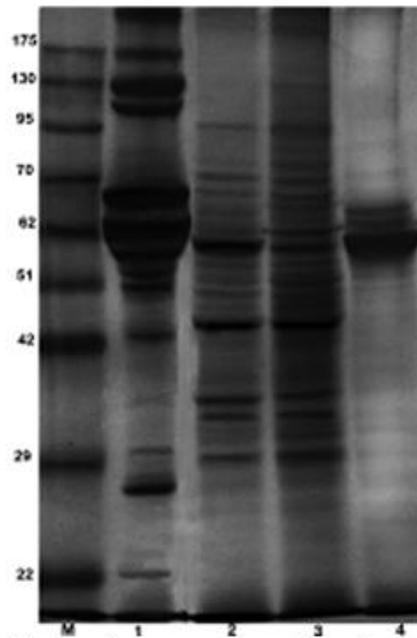


Fig. 3: SDS-PAGE analysis of soluble antigens of tachyzoites and bradyzoites of *T. gondii*, RH strain (cyst like structures) and bradyzoites of avirulent Tehran strain.

M, protein marker. Lane 1, tachyzoites of *T. gondii* RH strain. Lane 2, bradyzoites of *T.*

gondii RH strain (cyst like structures) developing in HeLa cells. Lane 3, bradyzoites of *T. gondii* avirulent Tehran strain. Lane 4, uninfected HeLa cells.

Discussion

In the present study, development of tachyzoites to bradyzoites of *T. gondii*, RH strain was demonstrated in continuous HeLa cell culture by PAS staining and SDS-PAGE in vitro. The mechanisms promoting tachyzoite to bradyzoite conversion in *T. gondii* are very complex and poorly understood. Some avirulent strains of *T. gondii* can produce tachyzoites and bradyzoites in cell culture (8, 9). Tachyzoite to bradyzoite conversion can be induced by applying external stress to various types of infected cell lines. There are reports of tachyzoite to bradyzoite conversion by pH changing of the culture medium (10). Basic pH (8.0 to 8.2) manipulations induce in vitro conversion of tachyzoites to bradyzoite (11, 12) as most researchers use the pH 8 treatments for this purpose (13).

In this study, cyst-like structures obtained by pH changing of the culture medium. The alkalization of culture medium to pH 8 induced expression of bradyzoite-specific proteins and production of RH cysts in cell culture (14). Cyst-like structures were noticed when they were stained with PAS staining method. The PAS method stains amylopectin granules in bradyzoites which are rarely seen in tachyzoites (15) but increase in bradyzoites (16).

SAG1, the tachyzoite-specific surface 30 kDa antigen is stage specific, being detected only in the tachyzoite, but absent in the bradyzoite stage. This antigen is abundant on the surface of tachyzoites (17, 18). Bradyzoites stop expressing of the tachyzoite-specific proteins (P30 and P22), but start expressing other antigens such P18, P21, P34 and P36 (19). In this study, the P34 and P36 were detected in cyst like structures of *T. gondii*, RH

strain by SDS-PAGE. The results of this study showed that cyst formation of *T. gondii*, RH strain for further studies on bradyzoites and conditions that affect the interconversion of bradyzoites and tachyzoites is possible in vitro.

Conclusion

Alkalization of culture medium to pH 8 could induce the expression of bradyzoite-specific proteins and production of RH tissue cysts in cell culture.

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