

MORPHOLOGICAL CHANGES IN A HUMAN RETINAL PIGMENT EPITHELIAL CELL LINE FOLLOWING INFECTION BY *Toxoplasma gondii*

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ABSTRACT

Retinitis is the most important clinical consequences of Toxoplasmosis but the mechanisms used by *T. gondii* tachyzoites to invade the retina are not yet understood. In the current study, cellular pathogenicity of Toxoplasmosis in a human retinal pigment epithelial cell line (ARPE-19) was investigated. Following *in vitro* infection of ARPE-19 with *T. gondii* tachyzoites, cell viability associated with infection was identified by Methyl thiazolyl tetrazolium (MTT) assay. Intracellular development of *T. gondii* tachyzoites within ARPE-19 was characterized by acridine orange (AO) staining. Morphological changes associated with infection was assessed by electron microscopy (EM). The results obtained showed that ARPE-19 permit the invasion, growth and development of *T. gondii* tachyzoites and that infection can cause a metabolic burden on the host cells and multiple morphological changes in the relocation of the host cell organelles (mitochondria, endoplasmic reticulum, and Golgi apparatus) around the parasitophorous vacuole

INTRODUCTION

T. gondii is an obligate intracellular pathogen that invades and proliferates within nucleated cells of warm-blooded animals. It is a major food-borne pathogen [1]. Infection of livestock creates a source of infection in humans [2]. Consumption of raw or undercooked meat is considered to be the major source of *T. gondii* infection [1,2].

In both human and animals, Toxoplasmosis causes reproductive failure, abortion birth defects, retinitis, iris, uveitis, keratitis, and hydrocephalus. However, *T. gondii* infection

is generally hidden and a symptomatic in the retina, brain, and other body tissues throughout life [3].

Retinitis is the most important clinical consequences of *T. gondii* infection because it's the most common site where tissue cysts are found [4,5]. The ability of intracellular pathogens to breach of the blood-retinal barrier(BRB) is key to their ability to cause retinitis [6]. This work aimed to identify the cell viability and characterise the structural changes in retinal pigment epithelial cells following infection by *T. gondii* tachyzoites.

MATERIALS AND METHODS

Cells

A human retinal pigment epithelial cell line (ARPE-19) were maintained in growth media consisting of Dulbecco's modified Eagle's medium (^cDMEM) supplemented with 10% foetal bovine serum and 100 U/ml penicillin and 100 µg/ml streptomycin. A trypan blue solution was used to quantify the number of viable cells present in the medium using a hemocytometer under a light microscope [7].

T. gondii

T. gondii tachyzoites (RH strain) were used in this study: *T. gondii* tachyzoites that were kindly provided by Professor Lee Innes (Moredun Research Institute, Scotland). They were propagated in the Madin-Darby Canine Kidney cell cultures in complete ^cDMEM [8].

Acridine Orange (AO) staining assay

ARPE-19 were grown on glass coverslips in 6-well plate at 1×10^5 cells/well. The cells were infected with *T. gondii* tachyzoites in a host:parasite ratio of 1:2. The cells were then washed twice in phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde, stained with 10% AO, rewashed in PBS, mounted onto a microscope slide, and examined using a Leica DM 5000B epifluorescence imaging system [7,8].

Methyl thiazolyl tetrazolium (MTT) assay

ARPE-19 were grown in 96-well plate at 5×10^3 cells/well. The cells were infected with *T. gondii* tachyzoites in a host:parasite ratio of 1:2. The cells were stained with 10 μ l MTT dye solution and incubated for 4h. Acidic isopropanol (100 μ l) was then added. Afterthat, cells were incubated for a further 1h and absorbance recorded using a microplate reader (Labtech International Ltd., UK) [9].

Scanning electron microscope(SEM)

ARPE-19 were grown on plastic coverslips in 24-well plate at 1×10^4 /ml. The cells were infected with *T. gondii* tachyzoites in a host:parasite ratio of 1:2. The cells were then washed, fixed with EM fixative buffers, dehydrated, dried, mounted on aluminum stubs, coated with gold-palladium, and observed under a SEM imaging system[8].

Transmission electron microscope (TEM)

ARPE-19 were grown on plastic coverslips in 24-well plate at 1×10^4 /ml. The cells were infected with *T. gondii* tachyzoites in a host:parasite ratio of 1:2. The cells were then washed, fixed with EM fixative buffers, dehydrated, infiltrated, and polymerized with resin. Resin blocks were sliced, stained with ethanolic urenyle acetate followed by lead acetate and examined using a TEM imaging system[8].

Statistical analysis

All data were analyzed using GraphPad Prism software, version 5.0 (Graph pad Software, Inc., San Diego, CA)

RESULTS

***T. gondii* replication in ARPE-19**

T. gondii tachyzoites replication following infection of ARPE-19 was identified at 48 h post infection(PI) using AO staining assay. Proliferation of tachyzoites had occurred and numerous tachyzoites were detected intracellularly (Figure 1 E, F, G, and H) compared to controls(Figure 1 A, B, C, and D).

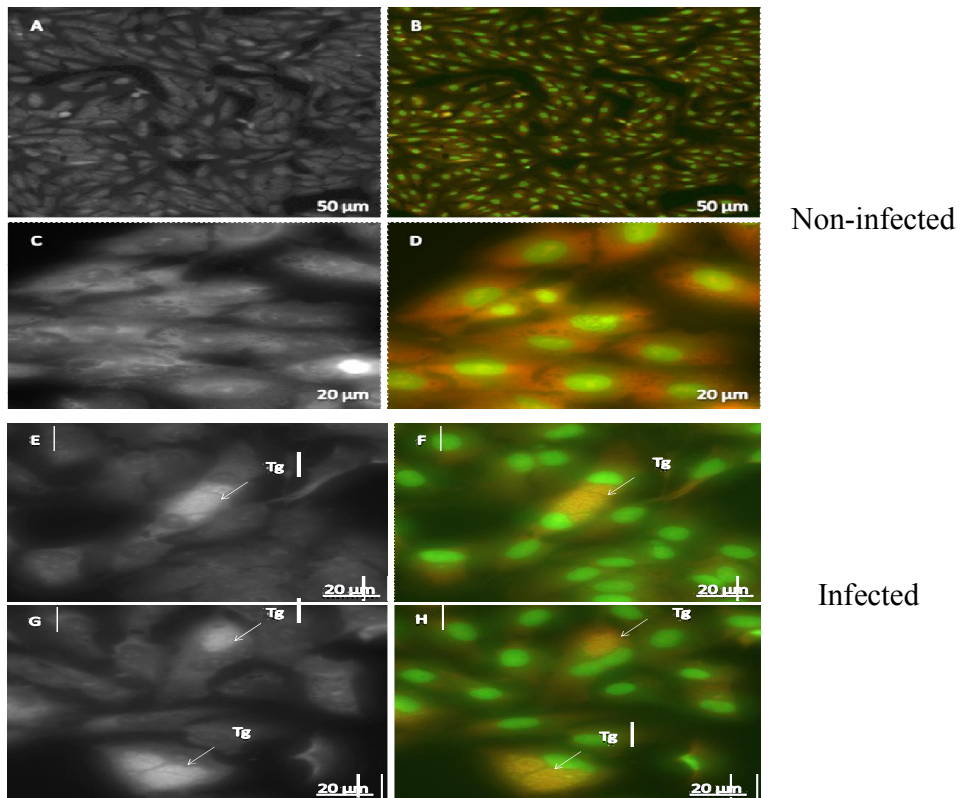


Fig.1. AO staining of infected cell showing development of *T. gondii* (Tg) tachyzoites compared to uninfected.

Epithelial cell viability

Viability of ARPE-19 was identified at 48 h PI using MTT assay. A significant decrease in the viability between the control and the infected cells had occurred ($P \leq 0.05$) (Figure 2).

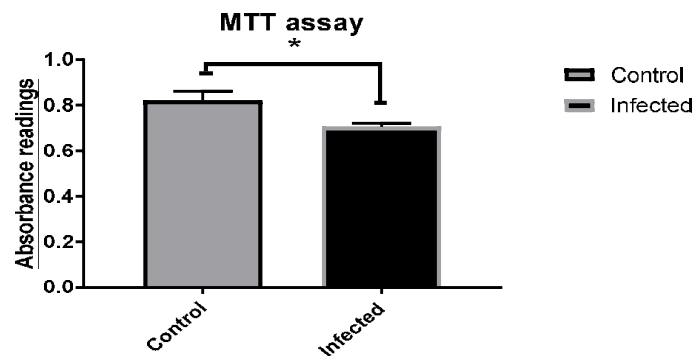


Fig.2. Viability of ARPE-19 showing a significant decrease between the control and the infected cells.

SEM imaging of infected cells with *T. gondii* tachyzoites

SEM showed the tachyzoites adhesion with the cells (Figure 3C) and the tachyzoites excited from the host cell when the host cell could no longer support the development of tachyzoites (Figure 3D). SEM also showed that tachyzoite proliferation damaged the tight junctions (TJs) (Figure 3C) compared to control (Figure 3 A and B)

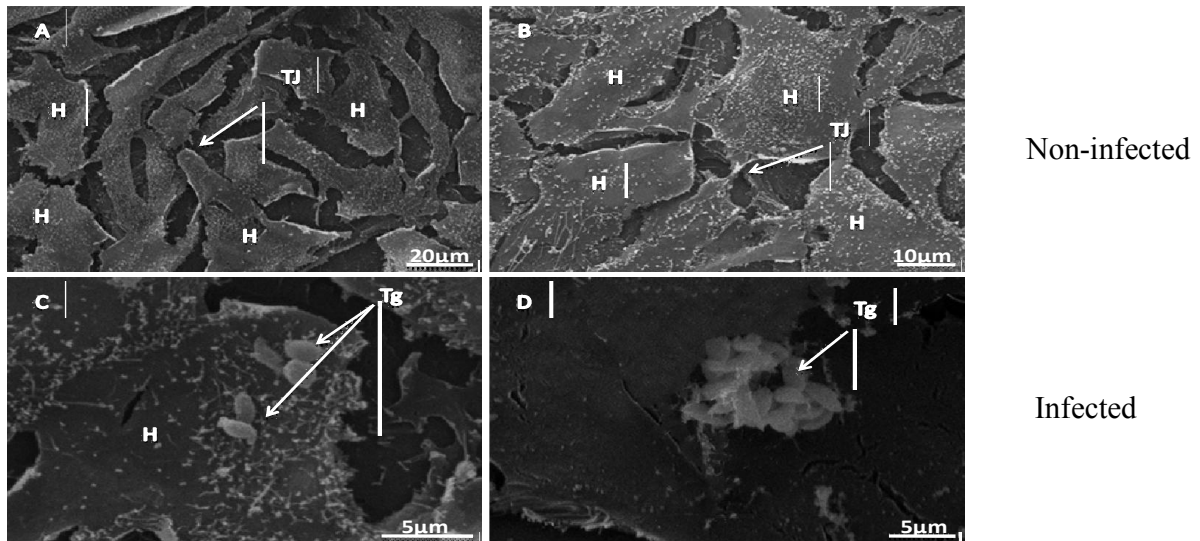


Fig.3. Proliferation *T. gondii* tachyzoites in infected cells. Electron micrographs showing the tachyzoites were adhered in the host cell (C). The exit of tachyzoites from the host cell was also noted(D). A large gap is obvious between infected cells (C) compared to control (A,B).

Ms showed tachyzoites in process of cellular division (Figure 4A). A parasitophorous vacuole (PV) and the parasitophorous vacuole membrane (PVM) is obvious (arrow) (Figure 4 B,C). TEMs also showed that host cell organelles concentrated around PV (Figure 4 D,E,F).

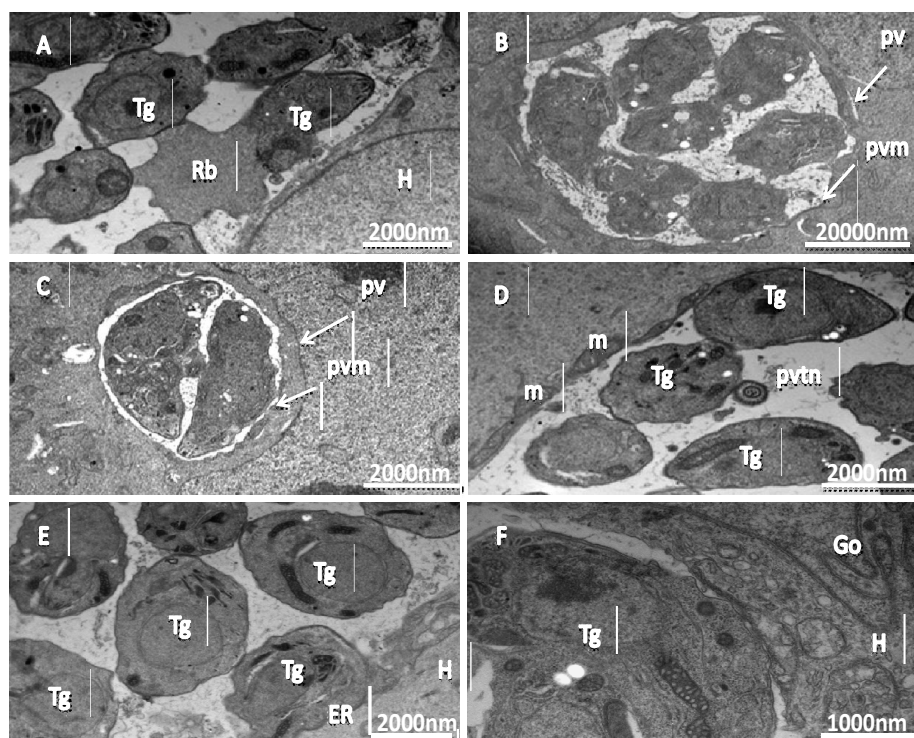


Fig.4. Replication of *T. gondii* tachyzoites in infected cells. Electron micrographs showing *T. gondii* tachyzoite (Tg) in the process of endodyogeny (A). A parasitophorous vacuole (pv) and parasitophorous vacuole membrane (pvm) is obvious (B,C). The host cell organelles (mitochondria, endoplasmic reticulum, and Golgi apparatus) concentrate around the parasitophorous vacuole (PV) (D, E, F).

DISCUSSION

The current study revealed that the retinal pigment epithelial cells can support development of *T. gondii*, by AO staining. This finding is in agreement with previous studies, in which the tachyzoites revealed a common pattern of growth and replication[7,8,10,11,12].

A marked decrease was noted in viability of infected cells compared to controls, by MTT assay, indicating that development of the tachyzoites caused a metabolic burden on the host cells. This finding is in agreement with previous study, in which the tachyzoites caused a metabolic burden on the host cells[8].

To highlight the morphological changes associated with infection, SEM and TEM were employed. Our observations using EM revealed that host organelles concentrate

around the PV throughout the development of the tachyzoites, suggesting the involvement of macromolecules integrated in the PVM for parasite development, membrane building, and energy requirement [13,14,15,16,17]. In addition, EM showed tachyzoites proliferate intracellular in the process of cellular division. This finding is in agreement with a previous study[18], where tachyzoites were reported to proliferate themselves in Vero cells intracellular in the process of endodyogeny. It has been found that tachyzoites release endosomal/lysosomal vesicles in the PV to siphon nutrient required for sustaining its replication [19,20]. Host cell microtubules and the tachyzoite protein, dense granule protein 7 (GRA7) have been implicated in this process[19]. In addition, rhoptry protein 2 (Rop2) and rhoptry protein 14 (Rop14) are involved in the recruitment of host mitochondria to the PVM and the composition of the PVM pore, respectively[19,21].

Our findings indicate that the invasion, growth, and proliferation of *T. gondii* tachyzoites within retinal pigment epithelial cells is possible, involving reorganization of the host cell organelles around the PV to support the parasite development and infection.

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دراسة التغيرات الشكلية للخلايا الطلائية لمبطنه لحاجز للعين عند الاصابه بطفيلي التوكسوبلازما

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الخلاصه

العين هي العضو الأكثر تضررا خلال عدوى طفيلي التوكسوبلازما لكن الميكانيكه المستخدمه من قبل الطفيلي لأصابه العين غير مفهومه الى الان. تم استقصاء الامراضيه الخلويه لطفيلي التوكسوبلازما في الخلايا الطلائيه المعزوله من حاجز العين . عقب اصابه الخلايا الطلائيه المعزوله من حاجز العين بطفيلي التوكسوبلازما في المختبر. قابليه الخليه للحياه تم تقديرها باستخدام اختبار ميثيل ثيازوليل تيترازوليوم باستخدام . تطور الطفيلي داخل

الخليه تم الكشف عنه باستخدام المجهر الفلوري. التغييرات الشكلية تم الكشف عنها باستخدام المجهر الالكتروني. اظهرت نتائج الدراسه الحاليه على قابليه طفيلي التوكوسبلازما على اختراق الخلايا الطلائيه المبطنه لحاجز العين والتضاعف داخلها وتجنيد محتويات الخليه (بيوت الطاقه، الشبكة الإندوبلازمية وجسيمات كولجي) حول الغشاء المبطن للكيسه التي يتواجد فيها التاكي زوئيت. كما اظهرت النتائج الحاليه ان تضاعف الطفيلي داخل الخليه شكل عبأ على الخليه المضيفه.

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