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Research Brief

One minute ultraviolet exposure inhibits *Toxoplasma gondii* tachyzoite replication and cyst conversion without diminishing host humoral-mediated immune response

Geetha Kannan [a,*], Emese Prandovszky [b], Curtis B. Steinfeldt [b], Kristin L. Gressitt [b], ChunXia Yang [a], Robert H. Yolken [b], Emily G. Severance [b], Lorraine Jones-Brando [b], Mikhail V. Pletnikov [a,c,d,e]

[a] Division of Neurobiology, Department of Psychiatry, Johns Hopkins University, 600 North Wolfe Street, Baltimore, MD 21287, USA
[b] Stanley Division of Developmental Neurovirology, Johns Hopkins University, 600 North Wolfe Street, Baltimore, MD 21287, USA
[c] Solomon H. Snyder Department of Neuroscience, Johns Hopkins University, 600 North Wolfe Street, Baltimore, MD 21287, USA
[d] Department of Molecular and Comparative Pathobiology, Johns Hopkins University, 600 North Wolfe Street, Baltimore, MD 21287, USA
[e] Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, 600 North Wolfe Street, Baltimore, MD 21287, USA

HIGHLIGHTS

- *T. gondii* tachyzoites exposed to UV for 1 min still have intact membranes.
- With 1 min UV exposure, tachyzoites are unable to replicate *in vitro* or produce parasite cysts *in vivo*.
- UV-inactivated tachyzoites can stimulate an IgG response but not cytokine response *in vivo*.

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ABSTRACT

We developed a protocol to inactivate *Toxoplasma gondii* (*T. gondii*) tachyzoites employing 1 min of ultraviolet (UV) exposure. We show that this treatment completely inhibited parasite replication and cyst formation *in vitro* and *in vivo* but did not affect the induction of a robust IgG response in mice. We propose that our protocol can be used to study the contribution of the humoral immune response to rodent behavioral alterations following *T. gondii* infection.

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1. Introduction

Toxoplasma gondii (*T. gondii*) infection is known to affect rodent behavior (Kannan et al., 2010; Lamberton et al., 2008; Vyas et al., 2007; Webster, 2001). While some behaviors, such as aversion to cat odor, are changed by all 3 *T. gondii* clonal subtypes (Ingram et al., 2013), other behavioral alterations, such as novelty-induced activity and learning and memory, are to some extent dependent upon the parasite strain (Kannan et al., 2010; Kannan and Pletnikov, 2012). It is unclear whether brain and behavioral alterations are due to a direct consequence of *T. gondii* cysts residing in the brain, or an indirect effect of the immune response to *T. gondii* infection. To discriminate the contributions of direct and indirect effects of *T. gondii* infection, experimental tools are needed to evoke an immune response to *T. gondii* proteins in the absence of active infection.

Tools to induce a *T. gondii* specific immune response without administration of a fully functioning live parasite already exist (Kur et al., 2009). For example, Toxovax®, a vaccine used to protect sheep, is made from a live attenuated Type I strain (Buxton and Innes, 1995). However, since each clonal lineage of *T. gondii* up-regulates cytokines in a strain-specific manner (Saeij et al., 2005), Toxovax® may not be suitable to study behavioral alterations specific

* Corresponding author. Address: Division of Neurobiology, Department of Psychiatry, Johns Hopkins University School of Medicine, CMSC 8-112, 600 North Wolfe Street, Baltimore, MD 21287, USA.
E-mail address: gkannan1@jhmi.edu (G. Kannan).

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to other *T. gondii* strains. Other vaccines made from single or combined purified *T. gondii* proteins (e.g. SAG1, SAG1 + GRA4) are able to stimulate an anti-*T. gondii* adaptive immune response (Kur et al., 2009), but the host immune response to single and/or denatured proteins may be different from the one induced by structurally intact tachyzoites. Consequently, we sought to develop a method to prevent *T. gondii* tachyzoite replication in the host without significantly altering the host's immune response to the parasite.

Ultraviolet (UV) irradiation has been shown to inactivate *T. gondii* tachyzoites. Current published protocols are based on a rather prolonged exposure to UV irradiation (i.e. up to 60 min) (Lu et al., 1999; Zhao et al., 2013), without clear demonstration that the inactivated tachyzoites are able to stimulate the host immune system in the absence of parasite replication or cyst production in rodent brains (Endo et al., 1981; Grimwood, 1980; Lu et al., 1999; Yang et al., 2010; Zhao et al., 2013). Here we describe a new rapid and effective method for UV inactivation of *T. gondii* tachyzoites in just 1 min. An advantage of our method is the use of standard equipment (Stratalinker®), thereby increasing the potential for reproducibility of the protocol. We demonstrate that 1 min of UV inactivation is sufficient to completely inhibit *in vitro* parasite replication and cyst formation in the mouse brain, while still leading to a robust humoral immune response in mice.

2. Materials and methods

Serology and *in vivo* parasite detection by PCR and cyst staining were performed using coded samples with the observer being unaware of their origin.

2.1. Parasite purification

Prugniaud (PRU) tachyzoites (⩽passage 3 *in vitro*) were cultured and purified as previously described (Kannan et al., 2010).

2.2. UV-inactivation of parasites

Purified parasites (30 μL) resuspended in Dulbecco's Phosphate Buffered Saline (DPBS) (1×) were added to an ultraviolet (UV)-transparent cuvette (Sarstedt AG & Co) and sealed with Parafilm®. The cuvette was placed horizontally in a UV Stratalinker® (STRATAGENE, UV Stratalinker 2400) and exposed to UV light for 1, 5, or 15 min at a power of 3689.04 μJ/cm^2/sec for a total energy exposure of 221,342.4, 1,106,712, or 3,320,136 μJ/cm^2, respectively. This was accomplished by setting the timer on the Stratalinker® for 60 min, which keeps the emitted power constant, and stopping the countdown after 1, 5, or 15 min. Control 0 min UV exposed parasites were added to a cuvette but not placed in the Stratalinker®. A schematic of the apparatus has been provided (Fig. 1).

2.3. Trypan blue exclusion

Live and UV-inactivated parasites were checked for damage to the cell membrane via the trypan blue dye test. Trypan blue solution (0.4% in PBS, Cellgro) was mixed with purified tachyzoites (1:1) and immediately visualized under brightfield using a Nikon Eclipse E400 microscope. Images were taken with the program EOS Utility 2.8.1.0 (Canon, EOS Rebel T1i camera).

2.4. Parasite replication assay

Purified live and UV-inactivated tachyzoites were examined for *in vitro* replication competency using a modification of an established immunofluorescence procedure (D'Angelo et al., 2009). Tachyzoites were added to HFF cells growing in 8-chamber slides (Millipore). At 2–9 days post-infection (37 °C, 5% CO_2), cell monolayers were rinsed with DPBS, fixed, permeabilized and then immunolabeled with rabbit (Rb) anti-SAG-1 (AbD Serotec, UK) followed by Alexa Fluor 594 goat anti-Rb (red, Life Technologies). DAPI (Invitrogen) for visualizing host cell nuclei was added to the secondary antibody. Stained cells were examined by epifluorescence using a Nikon eclipse E400 microscope with the program MetaVue version 6.2r6. Prior to staining, cells were examined by phase contrast using Axiovert 100 (Carl Zeiss) and images taken with Axiovision Rel 4.8.

2.5. Parasite red/green invasion assay

Purified live and UV-inactivated tachyzoites were examined for *in vitro* invasion competency as previously described (D'Angelo et al., 2009). Briefly, purified parasites were added to HFF cells growing in 8-chamber slides (Millipore). At 1 h post-infection, cells were rinsed, fixed and then immunolabeled with Rb anti-SAG-1. Cells were then permeabilized and immunolabeled with MAb 9e11 anti-SAG1 (Argene Inc., NY, USA) followed by a mixture of Alexa Fluor 594 goat anti-Rb (red, Life Technologies) to detect attached/extracellular tachyzoites and Alexa Fluor 488 (green, Invitrogen). DAPI (Invitrogen) was added to secondary. Cells were visualized via epifluorescence as described above.

2.6. Animals

Male BALB/c mice, 5 weeks old (The Jackson Laboratory, Bar Harbor, ME) were used in this study. Animal protocols were approved by the Animal Care and Use Committee of Johns Hopkins University (JHU). Mice were housed 3–5 per cage with 14.5/9.5 h of light/dark cycle and free access to food and water.

2.7. Mouse inoculations

Parasites were purified and UV-inactivated as described above. Alum adjuvant (Thermo Scientific) was added drop-wise to inactivated parasite (1:1) with mixing after each drop. Positive control innocula consisted of live parasites only. Negative control innocula consisted of DPBS and alum adjuvant (1:1). All solutions were placed on a rotating shaker (Barnstead Lab-Line) at medium–high speed for 40 min. Mice were injected intraperitoneally with 200 μL of either 400 UV-inactivated tachyzoites: alum adjuvant (n = 5; 2 tachy/μl; 1:1), 40,000 UV inactivated tachyzoites with and without alum adjuvant (n = 5; 200 tachy/μL; 1:1); 300,000 UV-inactivated tachyzoites with and without alum adjuvant (n = 5; 1500 tachy/μL; 1:1), 400 live tachyzoites (n = 4; 2 tachy/μL), or mock-infected with DPBS: alum adjuvant (n = 3; 1:1).

Boosters were administered 12 days post-infection. Mice first administered UV-inactivated tachyzoites with and without alum adjuvant or mock-infected with DPBS: alum adjuvant were given the same preparations as a booster. Mice first given live parasite were administered DPBS only as a booster.

2.8. Serology

Blood was collected via tail bleed and then centrifuged at 10,000*g* for 10 min on a table top centrifuge to separate cells from serum. Anti-*T. gondii* IgG was measured in sera using ELISA kits from IBL America (Minneapolis, MN, USA) modified as previously described (Xiao et al., 2009). The primary antibody consisted of diluted serum (1:100) and secondary antibody was enzyme labeled anti-mouse IgG.

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