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Original article

Toxoplasma gondii actively remodels the microtubule network in host cells

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Abstract

Toxoplasma gondii infection triggers host microtubule rearrangement and organelle recruitment around the parasite vacuole. Factors affecting initial stages of microtubule remodeling are unknown. To illuminate the mechanism, we tested the hypothesis that the parasite actively remodels host microtubules. Utilizing heat-killed parasites and time-lapse analysis, we determined microtubule rearrangement requires living parasites and is time dependent. We discovered a novel aster of microtubules (MTs) associates with the vacuole within 1 h of infection. This aster lacks the concentrated foci of gamma (γ)-tubulin normally associated with MT nucleation sites. Unexpectedly, vacuole enlargement does not correlate with an increase in MT staining around the vacuole. We conclude microtubule remodeling does not result from steric constraints. Using nocodazole washout studies, we demonstrate the vacuole nucleates host microtubule growth in-vivo via γ-tubulin-associated sites. Moreover, superinfected host cells display multiple γ-tubulin foci. Microtubule dynamics are critical for cell cycle control in uninfected cells. Using non-confluent monolayers, we show host cells commonly fail to finish cytokinesis resulting in larger, multinucleated cells. Our data suggest intimate interactions between *T. gondii* and host microtubules result in suppression of cell division and/or cause a mitotic defect, thus providing a larger space for parasite duplication.

Keywords: *Toxoplasma*; Protozoa; Microtubules; Microtubule organizing center; Host–pathogen interactions; Parasite vacuole

1. Introduction

Invasion of a host cell allows a pathogen to avoid various aspects of the immune system including antibody-mediated responses. This lifestyle however, requires development of strategies that exploit or manipulate the host in order to create an intracellular environment safeguarded from host defenses while maintaining accessibility to host nutrients. One of the most common strategies employed by intracellular pathogens is to modify the compartment in which they reside [1–3]. The apicomplexan parasite, *Toxoplasma gondii* alters trafficking within the host cell to deliver host vesicles and organelles [4–6] to the parasite vacuole membrane (PVM) for nutrient acquisition purposes.

Contact with host cells triggers adhesion and active penetration by *T. gondii*. Three specialized secretory organelles, micronemes, rhoptries and dense granules release their contents to aid in invasion and establishment of infection [7]. Micronemal proteins aid in adhesion, rhoptries contribute lipids and protein to vacuole formation, and dense granule proteins are important in modification and maintenance of the PV [1,6–9]. Host endoplasmic reticulum (ER) and mitochondria decorate the PVM [10,11]. The secreted parasite protein, ROP2, mediates adhesion of host mitochondria to the PVM [6,11,12] and functional microtubules are necessary for

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mitochondrial delivery to the *T. gondii* vacuole [11]. While functional MTs have been implicated in mitochondrial delivery and movement of PVs, MTs themselves were never directly visualized in these studies [5,11,12].

Few studies have directly assessed microtubule interactions with the PVM, the timing of MT remodeling, and the extent to which these interactions are the result of steric constraints. The studies outlined here address these issues. Interestingly, cytoskeletal rearrangement is not observed during parasite invasion [13] and this includes recruitment of host MTs around the *Toxoplasma* vacuole [14]. In contrast, other studies report host MTs decorate the PVM 4–6 h post-invasion [4,15,16]. Here we resolve the apparent discrepancy between these disparate observations by demonstrating recruitment does not become readily discernable until 1-h post-invasion.

A clearer picture of the role of MTs in nutrient acquisition has recently begun to emerge. Cholesterol scavenging from the host occurs via vesicular transport, and like mitochondrial delivery, requires functional host MTs [4,16]. Further, MTs and the host cytoskeleton filament vimentin, coat the PVM [4,17], and host microtubule-based invaginations of the PVM serve as conduits for host lysosome sequestration [4]. *T. gondii*, but not the related apicomplexan parasite *Neospora caninum*, triggers host MT rearrangement, indicating a parasite-specific phenomenon [4]. Building on this observation, we asked if MT recruitment requires active parasite intervention and identify several important factors that influence MT remodeling.

2. Materials and methods

2.1. Chemicals, antibodies and other reagents

All chemical reagents were purchased from Fisher Scientific, VWR or Sigma–Aldrich unless otherwise indicated. Fisher–Sigma–Genosys or Integrated DNA Technologies, Inc (Coralville, IA) provided primers. Restriction enzymes were purchased from New England Biolabs (Beverly, MA). All cell culture reagents were obtained from Gibco (Invitrogen, Carlsbad, CA). Antibodies were obtained from Sigma (monoclonal α-tubulin and polyclonal γ-tubulin antibody) and Molecular Probes (Alexa Fluor 488 goat anti-mouse secondary antibody IgG and Alexa Fluor 546 goat anti-rabbit secondary antibody IgG).

2.2. Host cells and parasites

RH tachyzoites and *N. caninum* were grown in the host cells (HFF) as previously described [18–20]. David Roos (University of Pennsylvania) generously provided the FNR-RFP cell line. *N. caninum* was a generous gift from Daniel Howe (MH Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky). Live cell imaging was performed in a stable BCS-1 line expressing GFP-tubulin at native levels and were maintained as previously described [21]. Macrophage studies were done with the RAW 264.7 macrophage-like cell line and cultured as previously described [22]. MaryAnn McDowell (University of Notre Dame) generously donated this cell line.

2.3. Microcopy

2.3.1. Fluorescence microscopy

Immunofluorescent assays (IFAs) were carried out as previously described [19] with the following modifications.

2.3.1.1. Wide field. Infected host cells (HFF) were fixed in methanol (at −20 °C) for 5 min and blocked in 5% BSA. Primary and secondary antibodies were both used at 1:1000. For heat-killed studies, parasites were subjected to a heat treatment of 56 °C for 3 h. Parasite viability was assessed with Trypan Blue (Cellgro, Mediatech, Inc.). All samples were viewed using a Leica DM IRE2, images were captured and processed using Openlab software as previously described [18,19].

2.3.1.2. Confocal microscopy. Samples were viewed using a Leica TCS SP2 (True Confocal Scanner) by Leica Microsystems. Instead of using filter sets, it uses an acousto-optical beam splitter (AOBS) crystal to separate the emissions wavelengths coming from the samples. The system is attached to a Leica DM IRE2 automated inverted microscope using a 100 W mercury bulb for fluorescence imaging, and the following objective lenses were used: 63× oil, and 100× oil. Confocal images were analyzed using ImageJ software (NIH) and the following plug-ins: Z-coded stack, Volume View and Maximum intensity projection [23,24].

2.3.2. Thin section transmission electron microscopy

Host cells were plated onto aclar discs rather than glass coverslips and infected with RH. The infection was allowed to proceed for 2 h at 37 °C, cells were mock-treated or treated with taxol (final concentration of 1 μM) and incubated an additional 2 h at 37 °C. The samples were then fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 1 h at room temperature, washed three times in 0.1 M cacodylate buffer and post-fixed for 1 h in 1.0% osmium tetroxide in the same buffer at room temperature. Samples were washed three times in water, stained for 1 h at room temperature in 2.0% uranyl acetate, washed in water and dehydrated in a graded series of ethanol. Subsequently, samples were embedded in Spurs, sections cut ultra thin at 80–90 nm, and viewed using a Hitachi H-600 transmission electron microscope.

2.3.3. Live image analysis

BCS-1 cell lines were plated on glass coverslips and grown for 48 h. They were subsequently infected with RH parasites and incubated for 16 h, washed twice with PBS and imaged live on the Leica DM IRE2 microscope as described above.