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A bradyzoite is a bradyzoite is a bradyzoite?

Kami Kim*

Departments of Medicine, Microbiology & Immunology, and Pathology, Albert Einstein College of Medicine, Bronx, New York, USA

Abstract

Bradyzoite forms of *Toxoplasma gondii* persist in tissue cysts for the lifetime of an infected host and can reactivate to cause clinical disease. It was thought that *in vivo* bradyzoites within tissue cysts are biologically inactive dormant forms that rarely replicate. Apparently, consensus was wrong.

Keywords

Toxoplasma; bradyzoite; tissue cyst

The opportunistic pathogen *Toxoplasma gondii* is an Apicomplexan parasite that has the unique ability to persist within its host as latent bradyzoite forms that lie within tissue cysts. The rapidly growing tachyzoite form that is responsible for clinical disease is controlled by the immune system and differentiates into bradyzoites. Bradyzoites have unique antigens and metabolism that are presumed to protect them from the immune response and facilitate long-term viability in tissue [1] (Figure 1). Bradyzoites are important because they can reactivate to cause lethal disease in an immunocompromised host. Although *T. gondii* can infect all nucleated cells, cysts are common in the brain, and clinical toxoplasmosis often presents as encephalitis, acute inflammation of the brain. Bradyzoites are also infectious when ingested. So the biology of bradyzoites has been the subject of intense interest to scientists investigating pathogenesis of toxoplasmosis.

Recently, Watts *et al.* took a closer look at the dynamics of *T. gondii* bradyzoites within tissue cysts [2]. After painstakingly optimizing the classic purification protocol developed by Cornelissen [3], Watts *et al.* examined the size, density, and bradyzoite contents of cysts harvested at different times from mouse brains. Ninety-nine cyst preparations, 630 cysts, and two years later, they found that more goes on within tissue cysts than had been appreciated previously.

Bradyzoite biology has been difficult to study. In the laboratory, bradyzoite differentiation can be induced by various environmental stresses, but it is difficult to obtain pure

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*Correspondence: kami.kim@einstein.yu.edu(K. Kim).

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populations of bradyzoites that are not contaminated with tachyzoite forms. Strains of *T. gondii* differ in their propensity to differentiate, and strains cultivated in the laboratory gradually lose their ability to differentiate. Epigenetic gene regulation also plays an important role in the tachyzoite-bradyzoite transition, but the exact molecular triggers for differentiation are not understood, as it has not been possible to follow the progression of bradyzoite differentiation over time. There has been controversy in the field whether laboratory induced bradyzoites are 'real' bradyzoites, with bradyzoites harvested from tissue cysts serving as the gold standard reference. It has been assumed that *in vivo* bradyzoites are homogeneous and static. The careful, standardized quantitation and characterization of cysts by Watts *et al.* [2] reveals unexpected heterogeneity amongst bradyzoites and tissue cysts.

Taking advantage of advances in three-dimensional digital confocal imaging, the authors developed the BradyCount software that enabled them to accurately and efficiently determine bradyzoite number within cysts. In general, as expected, as cyst size increased, the numbers of bradyzoites within cysts increased. They also carefully measured cyst size and used digital imaging approaches to quantify parasite division within cysts.

There was a surprisingly amount of variability in cyst density and bradyzoite burden within individual cysts, suggesting that cyst size varies independently of bradyzoite number over time. Bradyzoites are surrounded by a glycan-rich cyst wall, and the *Dolichos biflorus* agglutinin (DBA) lectin is a commonly used cyst wall marker. CST1, the major protein recognized by DBA lectin, is a glycoprotein that is extensively modified by O-linked sugars [4], and the glycosylation of CST1 confers rigidity to the cyst wall [4]. Since the size of cysts expands without replication of bradyzoites, the work by Watts *et al.* [2] suggests that the contents of the cyst matrix and components of the cyst wall are continuously secreted as cyst volume expands. How the cyst wall and cyst matrix are assembled is not yet clear, but one mechanism might be via ongoing secretion of glycoproteins analogous to the constitutive secretion of dense granule contents that occurs during tachyzoite development [5].

Numerous studies have shown the importance of parasite secreted molecules in interaction with the infected host cell and modulation of host signaling and transcription [6]. In addition, immunological studies show that bradyzoites within cysts are not as immunologically silent as originally thought, and are killed by both cytotoxic CD8+ T cells [7] and macrophages [8]. Intriguingly, it appears that alternatively activated macrophages recognize chitin in the cyst walls and produce chitinase that destroys cysts [8]. Therefore further understanding of the composition and dynamics of bradyzoite secretory pathways should be an area of fruitful investigation, and the glycoproteins induced during bradyzoite development may be new targets for vaccine or drug development.

Using IMC3, an intracellular marker whose expression is most intense in newly divided parasites [9], Watts *et al.* [2] also show that parasite replication does occur within cysts, and that this replication is not due to reversion of bradyzoites to tachyzoites. Bradyzoite replication was often asynchronous, but the authors also observed synchronous division, which is typically associated with the pathogenic tachyzoite form. Newly divided bradyzoites were found in clusters, uniformly through the cyst, as well as at the cyst

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periphery. The differing patterns of replication suggest that commitment to replication can be influenced by both local and external signals such as nutrients or small signaling molecules, as described in other microbial communities.

Since bradyzoites within tissue cysts are dynamic, we need to rethink our concepts about the biology of bradyzoites and strategies that might eradicate bradyzoites. Recent studies have reported compounds that reduce cyst numbers in brains [10]. While cyst number is an important parameter to monitor, quantitation of bradyzoite burden may be more important. Cyst number was relatively stable over the course of the experiments by Watts *et al.* [2], indicating that cysts that rupture or degenerate are replaced by an equal number of new cysts, and reinforcing the idea that ongoing immune surveillance is likely to keep chronic *T. gondii* infection in check. If agents are able to reduce the average number of bradyzoites, but reduce cyst number more slowly, we may need to reconsider the metrics used to assess efficacy of treatments to eliminate bradyzoites and cysts or modify dosing regimens to account for the biology and dynamics of cyst evolution. Further investigation is needed to understand the cyst maturation process and the associated metabolic and molecular changes. But it is clear that we can no longer think of *T. gondii* bradyzoites as a homogeneous quiescent population.

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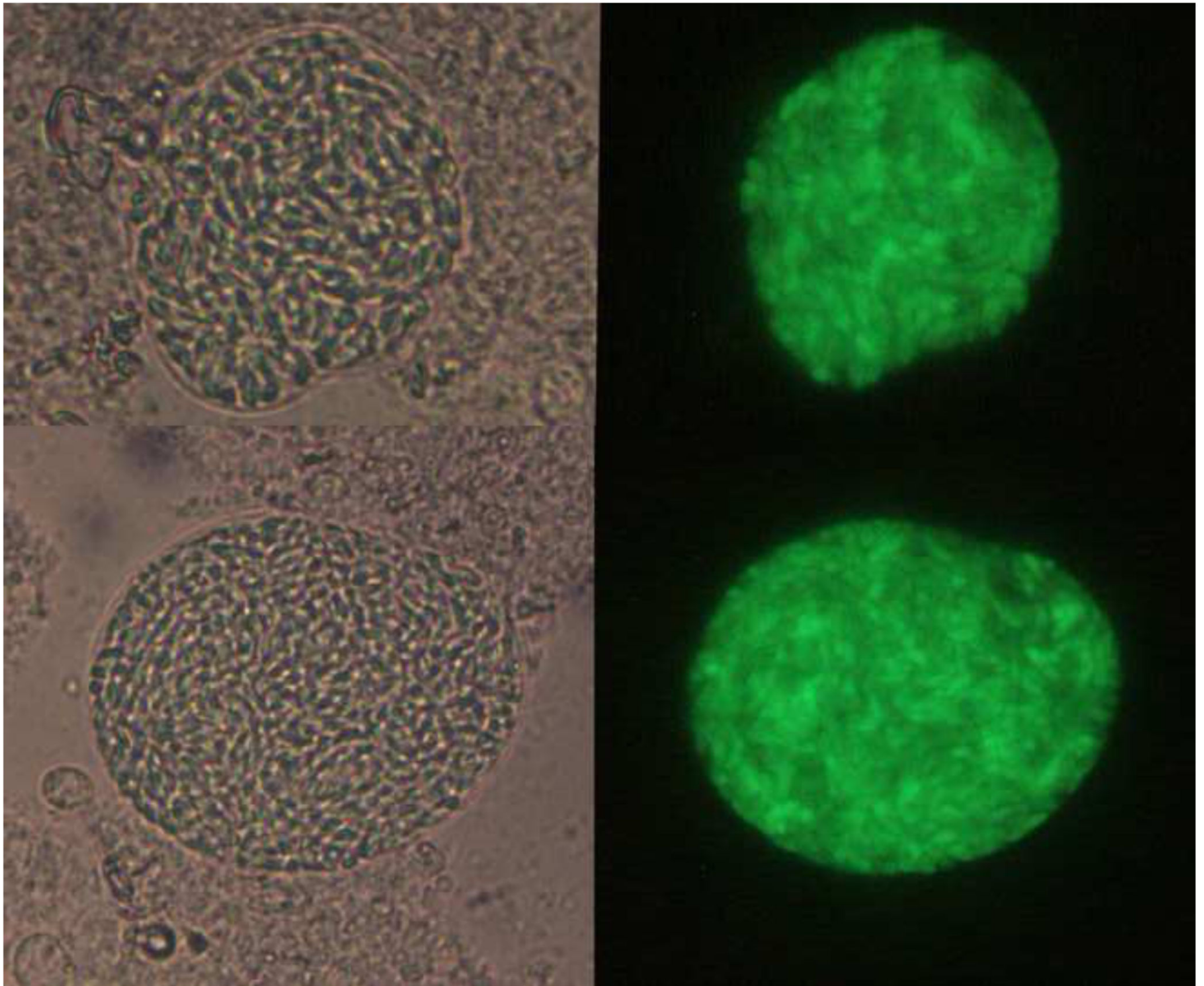

Figure 1. *Toxoplasma* tissue cysts harvested from mouse brains

The Pru *ku80 hxgprt* strain stably expresses GFP-positive cysts during chronic infection. The images show examples of cysts observed at 5 weeks after intraperitoneal infection of C57BL/6 mice with tachyzoites of strain Pru *ku80 hxgprtLDH2-GFP*. Left images show bright-field laser confocal microscopy and right images are GFP fluorescence driven by the bradyzoite-specific *LDH2* promoter. Images courtesy of Tadakimi Tomita and Louis Weiss.