

Wyoming INBRE

# Understanding iron regulation of *Toxoplasma gondii* dissemination during acute infection

Department of Molecular Biology

Allison Arp and Dr. Jason Gigley  
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## **Introduction**

The obligate intracellular protozoan *Toxoplasma gondii* (*T. gondii*) is a highly prevalent pathogen that infects approximately 30% of people world-wide (Tenter, Heckeroth, & Weiss, 2000). Although largely an asymptomatic infection, this parasite is a serious health threat for the developing fetus and those whose immune systems become compromised. To date there are no therapies or vaccines which provide thorough, sterilizing immunity to this pathogen. To develop these critical therapies and vaccines more knowledge is needed about parasite biology and the immune response generated during infection. The acute stage of infection is met with an immune response including IFN $\gamma$ , the most efficacious effector cytokine in host resistance to *T. gondii* (Scharton-Kersten et al., 1996). IFN $\gamma$  is known to control such effector mechanisms as activation of p47 GTPases, generation of reactive oxygen intermediates and nutrient starvation (Miller, Boulter, Ikin, & Smith, 2009; Silva et al., 2002; Sukhumavasi et al., 2008). In particular, IFN $\gamma$  is known to limit the availability of iron in a nutrient starvation effort common to the host response to many pathogens (Dimier & Bout, 1998; Gwamaka et al., 2012, 2012; Olakanmi, Schlesinger, Ahmed, & Britigan, 2002). Iron is a vital cofactor sequestered by the parasite from the host within infected cells (Miller et al., 2009). Based on published results from *in vitro* studies, iron sequestration is thought to inhibit parasite growth and replication. Importantly, iron can also impact the metabolism and resulting function of immune cells (Agoro, Taleb, Quesniaux, & Mura, 2018). However, when and how iron concentrations impact parasite dissemination and development of immune responses to this parasite remain unclear.

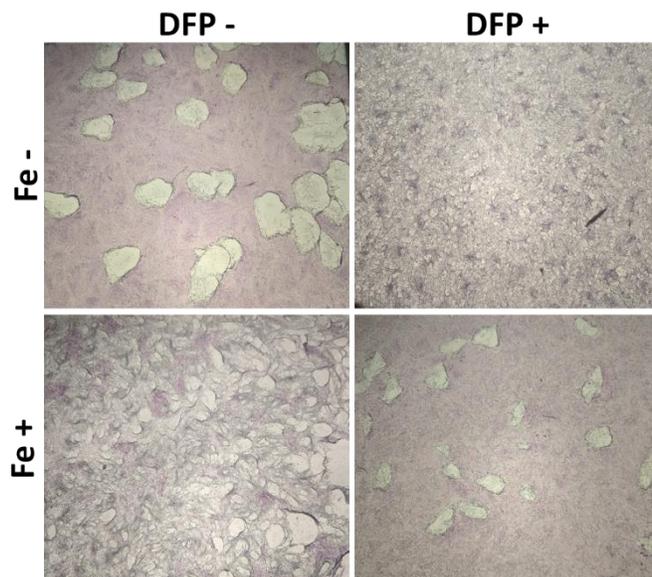
## **Methods**

*In vitro* assay. MRC5 human fibroblasts were obtained from ATCC and grown in T25 tissue culture flasks in complete DMEM. Once cells were confluent, the media was changed from complete DMEM to infection DMEM which contains 1 % FBS. Parasite were inoculated into the flasks with and without

Deferiprone and/or Ferric citrate. Parasites were allowed to grow for 7 days at which time plaques were visualized using crystal violet stain. Plaques were enumerated in each flask.

*Infection and Treatment.* Brains of infected mice were harvested and homogenized, and cyst counts were performed using a hemocytometer to assess cyst burden. Samples were then diluted to a concentration of  $1 \times 10^6$  cysts/mL and mice were infected intra-gastro-intestinally with 100 $\mu$ L of the solution. Mice receiving deferiprone treatment were administered 200 $\mu$ L of a  $1 \times 10^4$  solution of deferiprone the day prior to infection and every other day thereafter during the course of treatment.

*Organ preparation, DNA extraction, and real-time PCR.* Mice were sacrificed, and the spleen, liver, small intestine, lungs, and brain were harvested. Organs were digested with Qiagen proteinase K and AL buffer for 24 hours at 56 $^{\circ}$ C. DNA was extracted from the digested samples as per the Qiagen DNeasy Blood and Tissue protocol. Real-time PCR was performed using SYBR green detection and B1 primers to assess parasite number.



**Figure 1.** *In vitro* infection of human fibroblasts with RH. Treatment with deferiprone decreases plaque formation, and addition of iron restores it. Addition of iron without deferiprone treatment results in smaller, more frequent plaque formation.

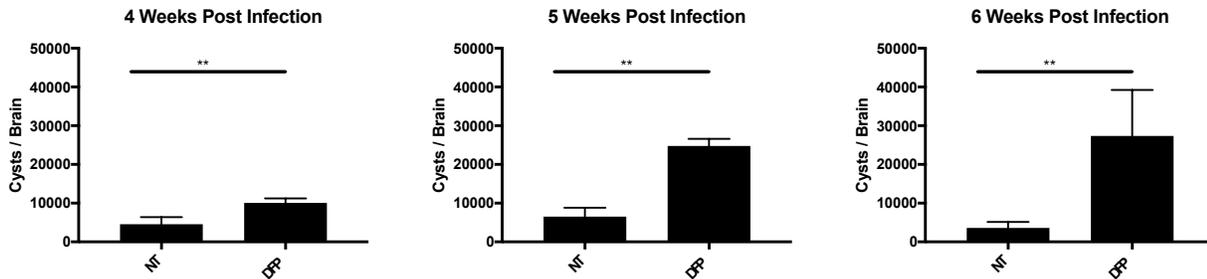
## Results

*In vitro* deferiprone treatment decreases plaque formation. To assess whether iron chelation via deferiprone decreases growth and replication of *T. gondii in vitro*, human fibroblasts were infected with RH. Assay results are shown in Figure 1. Infection with RH results in a large-plaque-forming phenotype. When treated with deferiprone, this phenotype dissipates in favor of less frequent, smaller plaques indicating a lack of

parasite growth and replication. Supplementation of iron following chelation via deferiprone treatment restores the large-plaque-forming phenotype. Congruently, supplementation with iron without administration of an iron chelator results in far greater lysis such that the plaques are not as distinct. The assay results suggest that iron is necessary for parasite growth and replication *in vitro*.

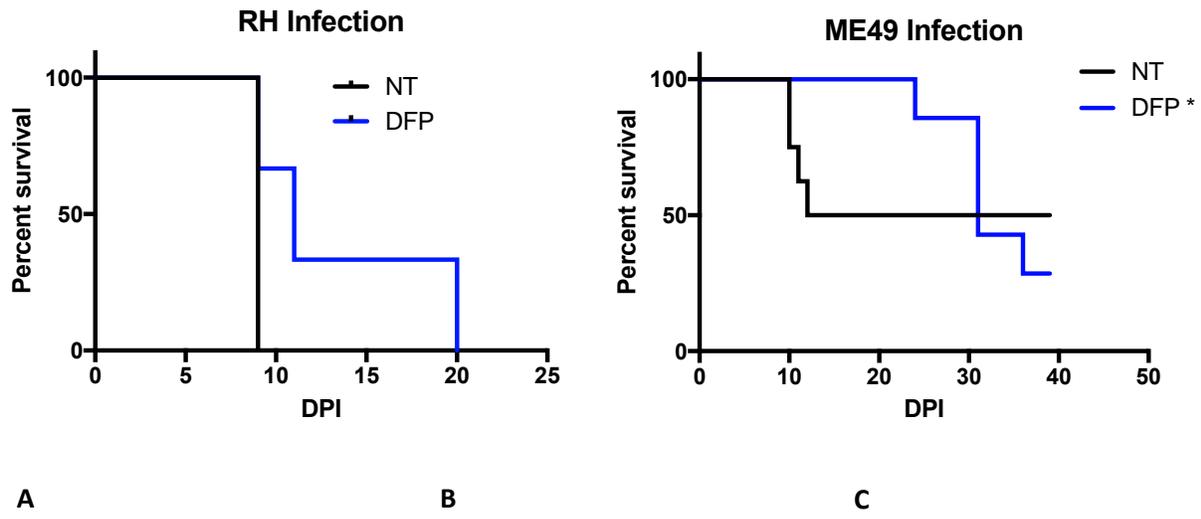
*Cyst burden increases with deferiprone treatment.* To complement the *in vitro* assay, *in vitro* measurements of *T. gondii* infection in the form of cyst burdens in the CNS were assessed. The brains of mice infected with ME49 were harvested four, five, and six weeks post-infection to assess the impact of deferiprone treatment on cyst burden during chronic infection. By four weeks, mice treated with deferiprone had significantly more cysts than untreated mice, and the gap increased from week four to week five and from week five to week six (Figure 2). This demonstration of increased *T. gondii* growth and replication directly contradicts the precedent set by the *in vitro* assay.

**Figure 2.** Cyst burdens in the brain with either deferiprone treatment, or no treatment 4, 5, and 6 weeks post-infection.



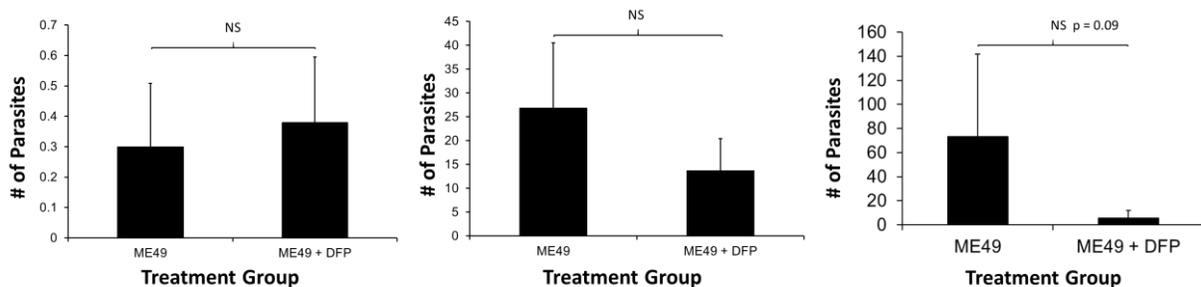
*Survivability following deferiprone treatment is increased.* Given that deferiprone treatment increases chronic cyst burden following ME49 treatment, the impact of said treatment on survivability of mice infected with either RH or ME49 was assessed. Though all of the mice infected with RH died and less mice infected with ME49 and treated with deferiprone survived than the control, both treated groups displayed a delay of death (Figure 3). Further, n=5 for all groups and repeats are expected to yield a more definite increase in survivability following infection with ME49. This delay of death supports the *in*

*vitro* precedent of decreased growth and replication of *T. gondii* following deferiprone treatment.



**Figure 3.** Survivability of B1 mice following infection with RH or ME49 with or without deferiprone

Treatment with deferiprone decreases dissemination of ME49 during acute infection. Given the conflicting *in vitro* and chronic cyst burden data, dissemination of *T. gondii* during acute infection with deferiprone treatment was assessed via parasite burden in the small intestine, spleen, and brain seven days post-infection. Deferiprone treatment decreased parasite burden at the site of infection (Figure 4C); although the difference is not significant, the data trends toward significance and repeats will push the p-value down. Similarly, parasite burden in the spleen trends down with deferiprone treatment (Figure 4B). No parasites were detected in the brain at seven days post-infection, as expected (Figure 4A).



**Figure 4.** Parasite burdens in mice seven days post-infection with ME49. Panel A is the brain, panel B is the spleen, and panel C is the small intestine.

## Discussion

As per the results of the *in vitro* assay, iron is essential for the growth and replication of *T. gondii* *in vivo*, a sensible result given the proclivity of the host response to include iron starvation (Dimier & Bout, 1998). Despite that, iron chelation via deferiprone treatment significantly increased chronic cyst burdens in the CNS, contradicting the *in vitro* precedent of parasite inhibition. This does not, however, negatively affect health outcomes; mice treated with deferiprone experience a delay of death despite increased chronic toxoplasmosis. Thus, the explanatory model of parasite dissemination during acute infection increasing with deferiprone treatment seems likely. That said, the impact of deferiprone treatment on *T. gondii* dissemination remains unclear. Parasite burdens at the site of infection trended toward significant reduction, and splenic parasite burdens trended down with deferiprone treatment in support of the *in vitro* precedent of impeded growth and replication. Ultimately, inhibition of parasite growth and replication due to deferiprone treatment is confirmed, but the mechanism linking said inhibition with increased encystation in the CNS remains unclear. These data support the hypothesis that iron is important for life stage conversion and that life stage conversion increases parasite dissemination.

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