Human brain microvascular endothelial cell-derived CCL2 drives *Toxoplasma gondii*-mediated BBB permeability

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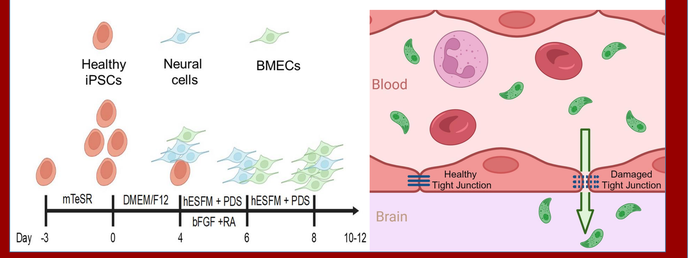
Background

Toxoplasma gondii is an obligate, intracellular protozoan parasite that is able to infect any nucleated cell of warm-blooded animals. In humans, *T. gondii* establishes a lifelong infection through the formation of cysts within neurons. The blood-brain barrier (BBB) is essential in limiting the movement of pathogens from the blood to the brain, and the mechanism by which *T. gondii* breaches the BBB, enabling lifelong cyst formation within neurons is not fully understood. The cytokines IFN-γ and CCL2 (MCP-1) are indispensable for host resistance against the parasite, and previous studies have shown them to be disruptive to the BBB. In our study we infected human induced pluripotent stem cell-derived BMECs with *T. gondii* tachyzoites, then in a temporal manner following infection we measured barrier integrity using trans-endothelial electrical resistance (TEER), sodium fluorescein (NaF) assays, and immunofluorescent microscopy (IF) to image ZO-1, Occludin, and Claudin-5 tight junction proteins.



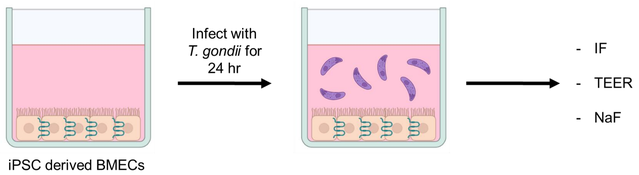
Hypothesis

- *T. gondii* infection mediates BBB permeability via tight junction de-localization.

Rationale

- *Toxoplasma gondii* is the causative pathogen of toxoplasmosis, and is one of the most common protozoan infections in the world
- More recent studies have shown latent *T. gondii* infection in humans to be associated with increased seizures and other neurological disorders such as schizophrenia and Alzheimer's disease.
- Discovering the method and mechanism by which *T. gondii* invades the parenchyma may unveil novel therapies in treating chronic toxoplasmosis.

Experimental Design



Results

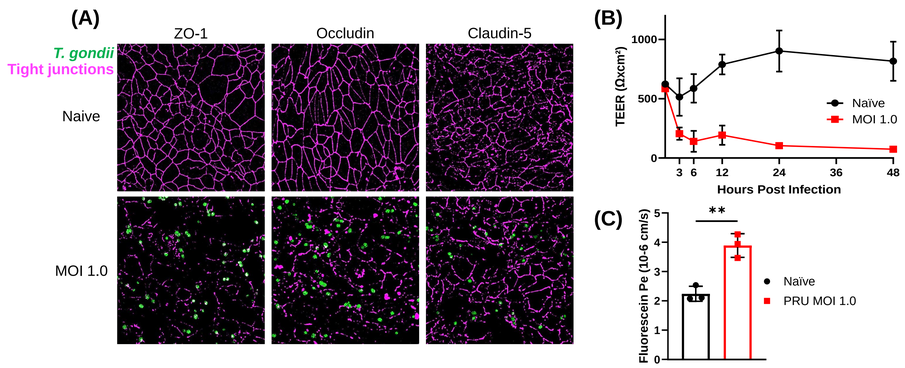


Figure 1. (A) iPSC-derived BMECs were immunolabeled for tight junction protein localization 24 hrs post *T. gondii* infection. BMECs were stained for tight junction proteins ZO-1, Claudin-5, and Occludin (magenta), and *T. gondii* (green). (B) TEER values were measured following *T. gondii* infection at 3, 6, 12, 24, and 48 hrs p.i. (C) Sodium Fluorescein values were measured at 24 hrs p.i. Statistics: unpaired parametric t-test, **P<0.01. Error bars, standard error mean (SEM). All tests were done in triplicate.

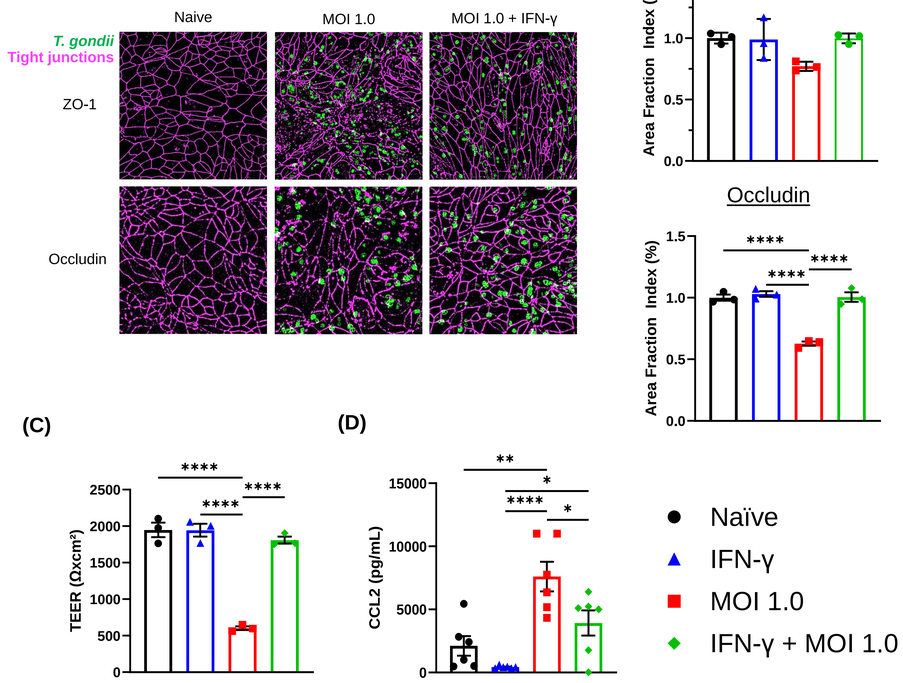


Figure 2. (A) iPSC-derived BMECs were immunolabeled for tight junction protein localization 24 hrs post *T. gondii* infection. 5.0ng/mL recombinant human IFN-γ was added to BMECs 24 hrs prior to *T. gondii* infection. BMECs were stained for tight junction proteins ZO-1, Occludin, and Claudin-5 (magenta), and *T. gondii* (green). (B) Quantification of area fraction index (%) was measured from immunofluorescent microscopy imaging as shown in A. (C) TEER values were measured at 24 hrs p.i. (D) CCL2 protein concentrations were measured 24 hrs p.i. using flow cytometry. Statistics: One-way ANOVA with post-Tukey, *P<0.05 **P<0.01, ****P<0.0001. Error bars SEM. All tests were done in triplicate.

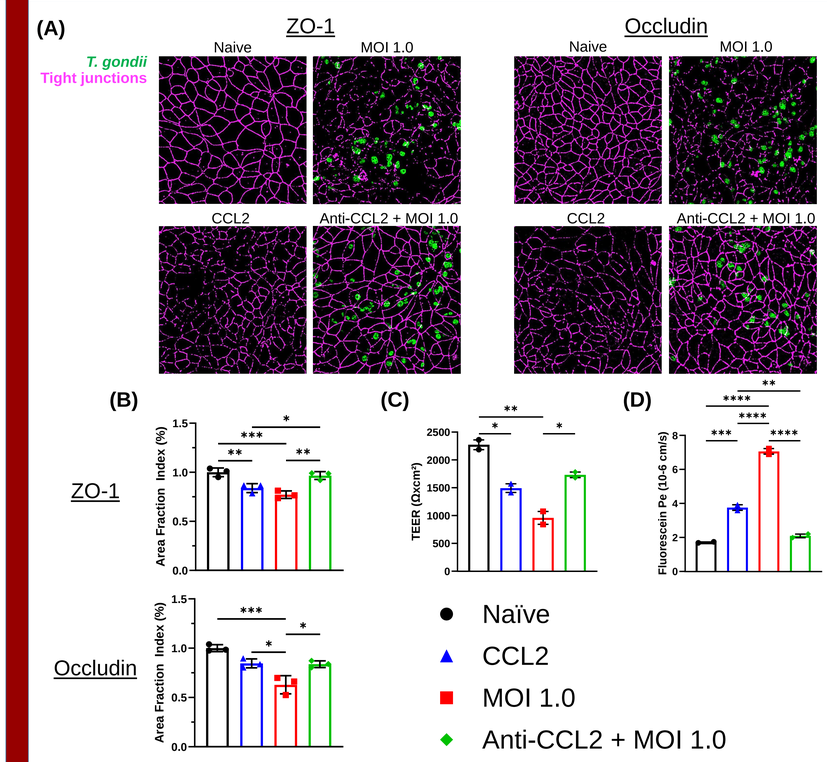
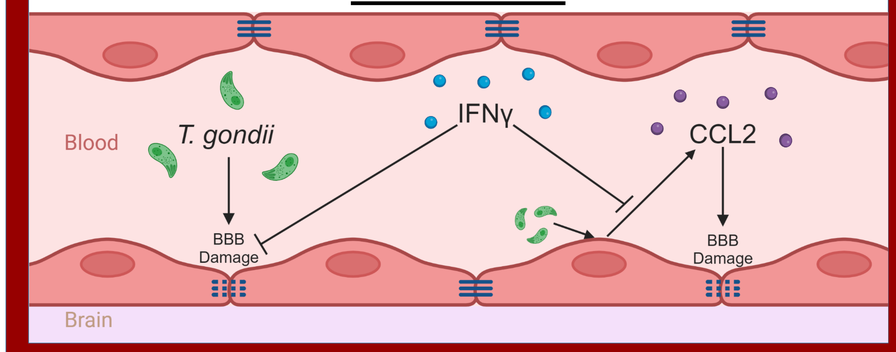


Figure 3. (A) iPSC-derived BMECs were immunolabeled for tight junction protein localization 24 hrs post *T. gondii* infection. 40μg/mL of recombinant human CCL2, or 100μg/mL of anti-CCL2 neutralizing antibody was added to BMECs at the time of infection. BMECs were stained for tight junction proteins ZO-1, Occludin, and Claudin-5 (magenta), and *T. gondii* (green). (B) Quantification of area fraction index (%) was measured from immunofluorescent microscopy imaging as shown in A. (C) TEER values were measured at 24 hrs p.i. (D) Sodium Fluorescein values were measured at 24 hrs p.i. Statistics: One-way ANOVA with post-Tukey, *P<0.05 **P<0.01, ***P<0.001 ****P<0.0001. Error bars SEM. Area fraction index done in triplicate, TEER and NaF done in duplicate.

Conclusions



Future Directions

- Investigate if *T. gondii* infection mediates increased CCR2 expression.
- Using a co-culture system, determine if IFN-g primed BMECs are sufficient to protect neurons from parasite infection.
- Further investigate the effects of IFN-g and CCL2 using *in vivo* cell type specific CCR2 deficient mice