The Isolation of Two Novel Parasite Genes (TGME49_269359 and TGME49_289050) Implicated in Chronic Toxoplasmosis

Introduction

Prevalence

- Toxoplasma gondii
  - Obligate intracellular category B priority pathogen
  - Single-celled parasite
  - Causative agent of toxoplasmosis
  - Neglected Parasitic infection targeted by CDC
  - Leading cause of death attributed to foodborne illness in the US
  - More than 60 million people in the United States carry T. gondii

Disease

- Immunocompetent individuals
  - Benign, flu-like symptoms
- Immunosuppressed individuals
  - Infants (congenital infection): miscarriage, stillborn, vision loss, seizures, mental disability, eye lesions
- AIDS patients: toxoplasmic encephalitis (cerebral lesions)
- Organ transplant recipients

Life Cycle

- Both the 68c6 and FIKK gene are important for tachyzoite conversion to cysts:
  - Evidenced by quantitative (cyst counts) and qualitative (photomicrographs) results
  - Can now be labeled as potential gene targets to disrupt cyst formation
  - The inability to convert to cysts may be due to:
    - Poor survival/replication in the face of a broad range of exogenous stresses
    - An inability to sense stress and respond appropriately with cyst formation
  - Mutant parasites are not sensitive to only one type of stress
  - Genes critical in a core pathway important for sensing exogenous stress, protection from stress, production and composition of cyst components

Reactivation

Results

- Both the 68c6 and FIKK gene are important for tachyzoite conversion to cysts:
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  - Can now be labeled as potential gene targets to disrupt cyst formation
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Research Goals

- Body response:
  - Cell mediated immunity
  - Endogenous nitric oxide
  - Unable to treat chronic infection
  - No current treatment for cyst establishment
  - Threat of reactivation still present

- Gene discovery project:
  - Two hypothetical genes (TGME49_269359 and TGME49_289050)
  - Gene roles in tachyzoite conversion to bradyzoites
  - Target to disrupt or eliminate cyst formation

Discussion

- Both the 68c6 and FIKK gene are important for tachyzoite conversion to cysts:
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  - Can now be labeled as potential gene targets to disrupt cyst formation
  - The inability to convert to cysts may be due to:
    - Poor survival/replication in the face of a broad range of exogenous stresses
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Future Research

- Complementation of gene deletions with respective WT genes
- In vivo studies
- Protein localization
- Hints to protein function
- Evaluate protein expression with endogenous tagging with yellow fluorescent protein and generation-independent cloning cassette

Gene Sequencing for TGME49_269350 gene

- RNA was isolated
- Primer sets for amplification of cDNA genes were designed
- Resulting cDNA used for gene sequencing and 3' end identification
- Purified DNA sequenced by GeneWiz
- Sequences blasted to ToxoDB database and assembled

Methods

- Parasites (Prugnaud strain) maintained on a monolayer of human foreskin fibroblast (HFF) cells
  - Cultured in a 5% CO2 incubator at 37°C
  - Chamber slides inoculated with 1 µl of parasites
  - Grown in HFF media for 3 days and exposed to exogenous stresses known to induce conversion of tachyzoites to cysts:
    - NO, alkaline pH, nutrient deprivation

Staining and Microscopy

- Fixed with 4% paraformaldehyde
- Chamber slides were permeabilized and stained for immunofluorescence [rabbit polyclonal anti-Toxoplasma Abs, Alexa Flour 546 goat anti-mouse, FITC-conjugated Dolichos biflorus agglutinin (DBA) lectin]
- Chamber slides were mounted
- Fully formed cysts were counted per 100 parasites within chamber slides under 100x
- Photomicrographs were taken using identical resolutions for each cyst

Bibliography

5. CST1, a Toxoplasma gondii cyst wall glycoprotein. Infection and immunity 69: 501 - 507
8. Fikk KO Cyst Counts

Summary: Deleted genes have been identified as important for parasite survival in response to stress. Poor survival leads to inherent vulnerabilities that future therapeutics can be designed to target in order to prevent life threatening infection.