MOI Project 16

Function of mGBP protein complexes in cell-autonomous immunity against *Toxoplasma gondii*

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Background

The immune recognition process is orchestrated by pattern recognition receptors (PRRs) that detect invading pathogens. This triggers a cascade of events leading to cytokine production, which are critical signaling molecules that regulate immune responses. Among them, interferon-gamma (IFNγ) plays a key role in activating and modulating immune responses to control infections by inducing antimicrobial functions in both immune and non-immune cells. IFNγ stimulates the activation of interferon-stimulated genes (ISGs), including interferon-inducible GTPases. These GTPases, such as Guanylate-binding proteins (GBPs), play a crucial role in vertebrate cell-autonomous immunity. Particularly, GBPs are effective antimicrobial effectors against intracellular pathogens like *Toxoplasma gondii*, a clinically significant parasite. In mice, certain GBPs are highly expressed following IFNγ stimulation and target pathogen-containing compartments, leading to the destruction of the parasitic vacuoles. Knockout studies in mice further confirm the critical role of GBPs, especially mGBP7, in host defense against intracellular parasites.

Previous Work

To explore the molecular interactions and regulatory mechanisms of mGBP7, we identified several binding partners, including TOM1, a cytoplasmic protein known to interact with clathrin and Tollip, and to bind ubiquitinated proteins. We found that TOM1 localizes to the *T. gondii* parasitophorous vacuole (PV), similar to mGBP7. Conditional TOM1$^{-/-}$ mice have been generated for further characterization to understand the antiparasitic effects of mGBP7. Additionally, we created an mGBP9$^{-/-}$ mouse line. mGBP9, part of the largely unexplored chromosome 5 mGBP family, showed increased susceptibility to *T. gondii* infection in initial experiments. Preliminary data suggest mGBP9 may play a role in inflammasome activation, which will be investigated further in this project.

Aim of the project

This project aims to further characterize the intracellular, cell-autonomous response of mGBPs and their interacting molecules to *T. gondii* infection, enhancing the understanding of host-pathogen interactions in parasitic infections.

Work program

As part of this project, the following tasks are planned:

- Characterize the role of TOM1 in *T. gondii* infection by infecting TOM1$^{-/-}$ mice and analyzing survival, parasite load, and inflammation markers.
- Conduct *in vitro* studies to measure *T. gondii* proliferation using a luciferase-expressing strain in WT and TOM1$^{-/-}$ cells and examine mGBP recruitment efficiency in the absence of TOM1.
- Investigate the roles of Tollip and clathrin, known TOM1 interaction partners, in *T. gondii* infection through microscopy and functional studies.

- Investigate the role of Tollip in recruiting galectins to intracellular pathogen vacuoles and *T. gondii* PV, using a Crispr/Cas9 screen to further explore galectin functions in *T. gondii* infection.
- Expand studies on mGBP9-deficient mice, which show susceptibility to *T. gondii* infection, and compare results to TOM1$^{-/-}$ mice.
- Use the Proximity Labeling method to identify mGBP9 interaction candidates in the cytoplasm and at the parasitic vacuole.