Using in vivo bio-luminescence imaging and FACS based approaches to understand the role of *Nfkbid* in immunity to *Toxoplasma gondii*

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Introduction

Toxoplasma gondii (T-gondii) is one of the most common forms of intracellular parasites in the world, infecting nearly one-third of all humans. The single-celled parasite is the causative pathogen for toxoplasmosis, an orally acquired infection that affects the brain, heart, and skeletal muscle of a host. Toxoplasmosis represents one of many neglected parasitic diseases that are not only a major public health issue but also lack an effective vaccine.

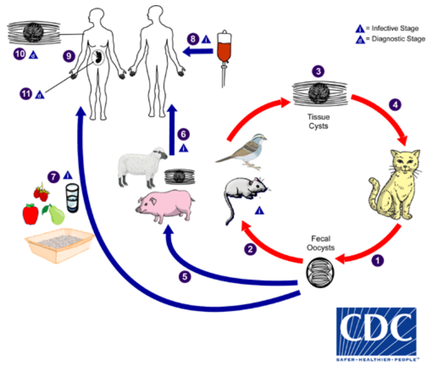

Figure 1. Life cycle of *Toxoplasma-gondii*

Background

- Ongoing research has been focused on understanding host-pathogen interactions of *T-gondii* as it relates to the susceptibility of a host to parasitic infections and how the host develops immunity
- Host gene *Nfkbid* was required for secondary immunity against virulent strains of *T-gondii*
- Mice without *Nfkbid* (bumble) had a lower survival rate and failed to make IgM and little IgG which are key antibodies that recognize and bind to *T-gondii*

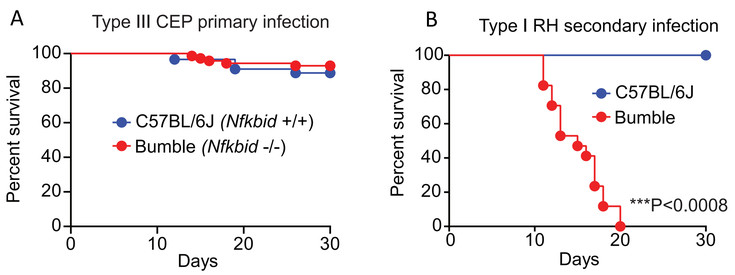

Figure 2. Survival curves of bumble (*nfkbid-/-*) and WT (B6) mice against primary (CEP) and secondary (RH) **A.** Shows that *Nfkbid* knockout mice survive vaccination at the same rate as the parental wildtype strain (B6). **B.** mice without *Nfkbid* are unable to survive high virulence strains of *T. gondii.*

Objective

To investigate the effect of *Nfkbid* in immunity to *Toxoplasma gondii*

Methods/Rationale

Antibody Reactivity

I hypothesize that mice with lower *Nfkbid* will possess less-parasite specific antibodies than mice with normal *Nfkbid* expression.

- Flow cytometry is the process measuring and analyzing multiple characteristics of single cells or particles as they pass through a beam of light in liquid suspension.
- Fluorescence activated cell sorting (FACS) data were analyzed from three different mouse strains; AJ, B6, and heterozygous Bumble. The samples consisted of parasites coated with serum collected from infected mice with *T-gondii*. The samples were analyzed for the presence of parasite-specific antibodies, detected by antibodies specific to individual mouse isotypes.

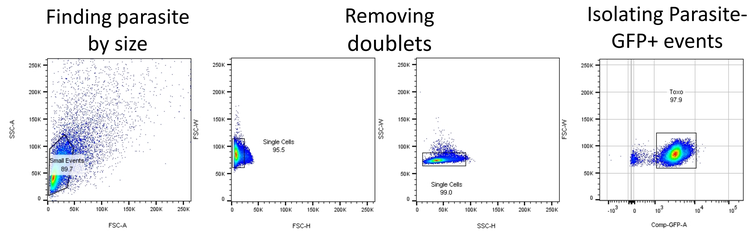

Figure 3. Gating strategy of serum coated GFP+ parasites

In vivo bioluminescence imaging

I hypothesize that bumble (*Nfkbid* knockout) will greater parasite burden than WT B6 mice.

- Utilizing In vivo bioluminescence imaging (BLi), mice will be infected with a luciferase-expressing parasite along with Luciferin as the bioluminescent substrate.
- Emitted photons per second, photon flux, will be detected by a single photon detection camera. The photon flux will correlate with parasite burden
- Mouse pictures will be overlaid with collected photon emission images to demonstrate the concentration of the parasite within each mice. The process will be repeated every 3-4 days for a total of 30 days.
- Mice with a single gene altered, B10.A, showed less photon flux compared to B10 mice, which is correlated with a lower parasite burden. This is a proof of concept that changing one gene can dramatically alter a host's response to *T. gondii.*

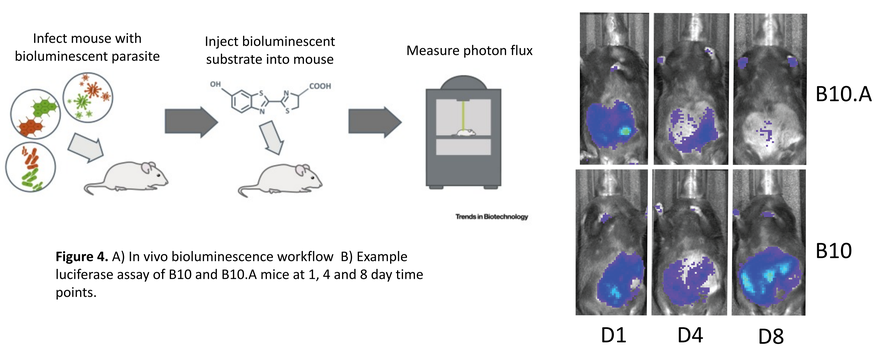

Figure 4. A) In vivo bioluminescence workflow B) Example luciferase assay of B10 and B10.A mice at 1, 4 and 8 day time points.

Results

The results of the FACS data analysis demonstrate a difference between the immune response of mice with deficient (heterozygous bumble) and normal *Nfkbid* (WT) expression. In the experiment each fluorophore is associated with a specific antibody isotope; IgM-PECy7, IgG1-APC, IgG2b-PE, IgG2a-PerCPCy5.5, and IgG3-BV421. B6 samples had relatively less PE, BV421, APC, and PerCP-Cy5-5 fluorescence than heterozygous bumble. The negative control sample, BN, illustrates that this is not a result of any false positive signaling. The results show not only that mice with lower *Nfkbid* possess less parasite-specific antibody but produce different amounts of specific-antibody isotypes. In this case, B6 mice produced less IgG2b, Igg3, IgM, and IgG3.

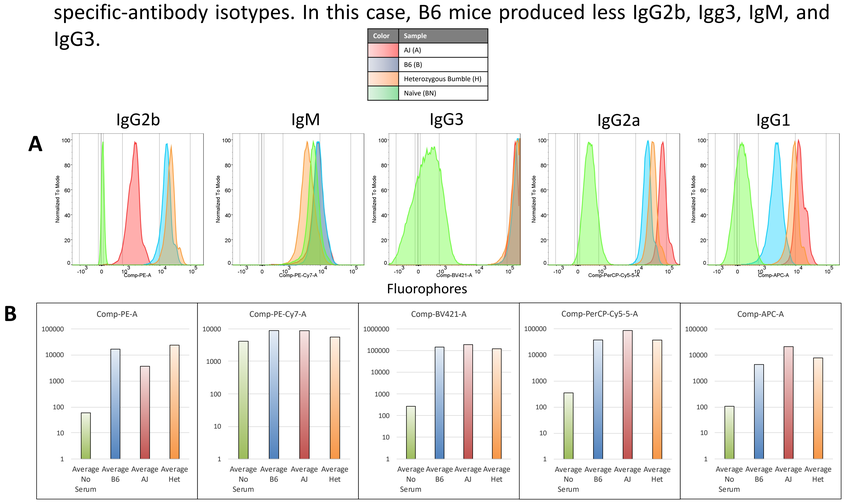

Figure 5. A. Associated antibody isotype by histogram for each dilution (IgG1 is APC, IgG2a is PerCPCy5.5, IgG2b is PE, IgG3 is BV421, and IgM is PECy7) for AJ, B6, Heterozygous bumble (H) and negative control (BN2) **B.** Cumulative parasite-specific antibody MFI

Conclusions

The expectation is that there will be some difference between the immune response between B6 and Bumble mice. Our FACS results demonstrate that mice with reduced *Nfkbid* do produce different amounts and types of antibody's isotypes against the parasite. But, The FACS results do disprove my initial hypothesis that bumble het would have a less relative amount of antibodies than B6 mice. Nevertheless, These results suggest that *Nfkbid* is controlling the parasite specific-antibody isotypes produced by a host immune response. We expect bumble mice to have greater parasite burden than B6 mice. This conclusion will be supported by In vivo bioluminescence imaging that should display greater photon flux from bumble mice.

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References

CDC - DPDx - Toxoplasmosis. (2017, December 18). Retrieved July 20, 2020, from https://www.cdc.gov/dpdx/toxoplasmosis/index.html