

# The role of Complement Receptors (CR) 1 and 2 in *Borrelia burgdorferi* infection

**The role of Complement Receptors (CR) 1 and 2 in *Borrelia burgdorferi* infection**  
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**Introduction**

- Lyme Disease, the most common vector-borne disease in the US, is caused by *Borrelia burgdorferi* (Bb).
- Strain-level heterogeneity in Bb during early inflammatory responses facilitates chronic disease.
- Immune loads (i.e. total) diverge persistently, non-resolving infections with variable responses.

**Figure 1: Bb Strain and Hosts.** Bb is a spirochete bacterium that is transmitted via Ixodes ticks between hosts.

**Exploring Mechanisms of *Borrelia burgdorferi* Persistence in Host**

**Hypothesis:** Bb regulates complement activation and has subtle complement evasion responses.

- Multiple proteins of Bb suppress complement activation and has subtle complement evasion responses.
- BBK32, a protein expressed by Bb, is a known inhibitor of the classical complement pathway.
- Complement also regulates the adaptive immune response through the presence of complement factors with complement receptors CR1 and CR2.

**Complement Receptors**

**Methods**

- Flow cytometry to quantify immune cell populations at day 28 post-infection in the draining lymph node.
- qPCR to measure Bb *Borrelia burgdorferi* tissue load.
- ELISA serum analysis to measure anti-Bb IgG responses.

**Aim 1: Study the effect of CR2 expression by Bb on the basis of immune response to Bb infection.**

- Crude preparations using mixed bovine receptor (BR) vesicles obtained generated by lysis of modulation of CR2 (CR2<sup>+/+</sup>) were followed by infection.
- BR vesicles from BR:WT mice → CR2<sup>+/+</sup> and delivered Bb were → all cells express CR1 and CR2.
- BR vesicles from BR:IM from Bb:CR2 deficient mice → CR2<sup>+/+</sup> mice → CR2<sup>+/+</sup> deficient mice → Bb cells are detected in CR2:CR2 (best cells see overall).

**Aim 2: Study the effects of BBK32 delivery on Bb and responses to Bb.**

**Methods**

- Flow cytometry to quantify immune cell populations at day 28 post-infection in the draining lymph node.
- qPCR to measure Bb *Borrelia burgdorferi* tissue load.
- ELISA serum analysis to measure anti-Bb IgG responses.

**Aim 3: Study the effects of BBK32 delivery on Bb and responses to Bb.**

**Methods**

- Crude preparations using WT mice infected with:
  - WT Bb *Borrelia burgdorferi*
  - BBK32 KO Bb *Borrelia burgdorferi*
  - BBK32 supplemented Bb *Borrelia burgdorferi*

**Discussion**

- Hypothesis that knocking out a receptor required for complement activation shows no effect. Results would suggest that:
  - CR2 antibodies may not be an immune evasion strategy utilized by Bb.
  - CR1 antibodies may be an strategy inhibited by Bb that no changes would be observed.
- By studying immune evasion strategies we expect to help the development of future therapeutic interventions that support pathogen clearance.

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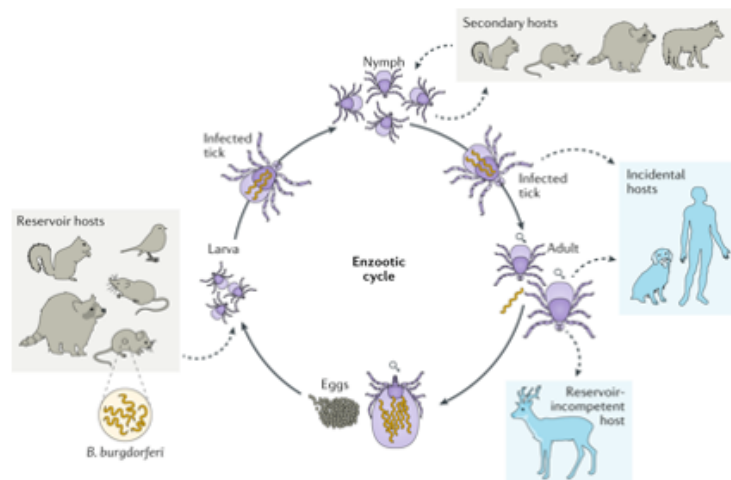
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## INTRODUCTION

- Lyme Disease, the most common vector-borne disease in the US, is caused by *Borrelia burgdorferi* (Bb).
  - Incidental hosts (i.e. humans) develop strong inflammatory responses that lead to chronic disease.
  - Reservoir hosts (i.e. mice) develop persistent, non-resolving infections with mild/no symptoms.

**Figure 1:** Bb lifecycle and hosts. Bb is a spirochete bacteria that is transmitted via bites from infected ticks.



Kurokawa, C., Lynn, G.E., Pedra, J.H.F. et al. Interactions between *Borrelia burgdorferi* and ticks. *Nat Rev Microbiol* **18**, 587–600 (2020). <https://doi.org/10.1038/s41579-020-0400-5>

- Immediately, strong antibody responses are generated after Bb infection.
  - Ongoing hypergammaglobulinemia helps control bacterial load but host cannot clear infection.
- T cell-dependent germinal center (GC) responses rapidly collapse and generate neither long-lived plasma cells, nor memory B cells.
- Mechanisms underlying this lack of antibody effectiveness are unknown.



# EXPLORING MECHANISMS OF BORRELIA BURGdorFERI PERSISTENCE IN HOST

**Hypothesis:** *B. burgdorferi* interferes with the normal function of the complement system. This inhibition suppresses an effective antibody-mediated immune response.

- Multiple proteins of Bb suppress complement activation and thus inhibit complement-mediated lysis.
  - BBK32, a protein expressed by Bb, is a known inhibitor of the classical complement pathway.
- Complement also regulates the adaptive immune response through the interaction of complement factors with complement receptors (CR) 1 and 2.
  - CR1 (CD35) & CR2 (CD21) are located on cells within the germinal center i.e. B-cells and are important for antigen presentation.

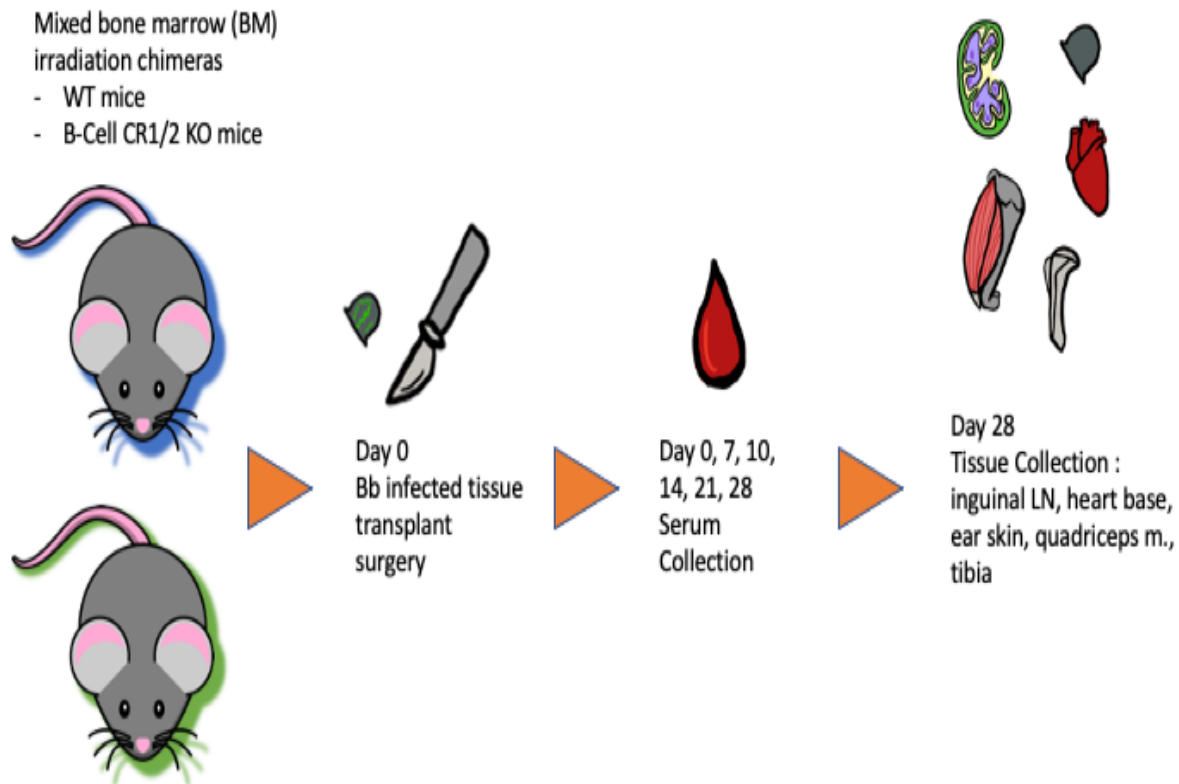
# COMPLEMENT RECEPTORS

## Methods

- Flow cytometry to quantify immune cell populations at day 28 post infection in the draining lymph node
- qPCR to measure *B. burgdorferi* tissue load
- ELISA serum analysis to measure anti-Bb IgG responses

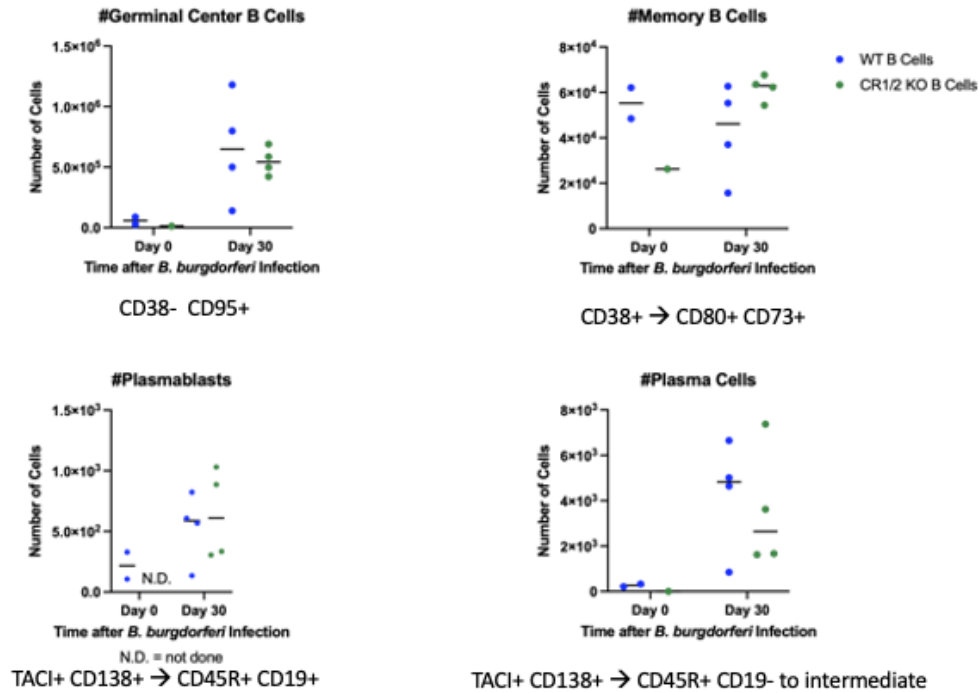
**Aim 1:** Study the effect of CR1/2 expression by B-cells on humoral immune response to Bb infection.

- *In vivo* experiments using mixed bone marrow (BM) irradiation chimeras generated by lethal irradiation of C57BL/6 (wild type) mice followed by either:
  - BM replacement from 80% WT mice + 20% B cell deficient WT mice = **all cells express CR1 and CR2**
  - BM replacement from 80% BM from B-cell deficient mice + 20% BM from CR1/CR2 deficient mice = **B-cells are deficient in CR1/CR2 (most cells are normal)**

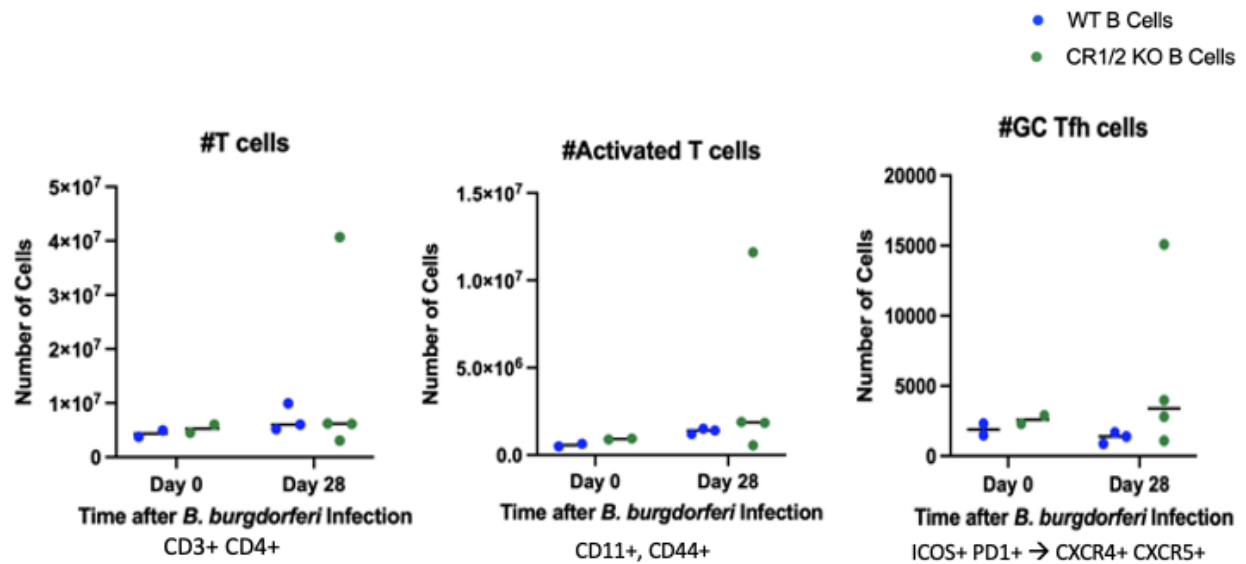


# AIM 1 RESULTS

**Figure 3:** Flow cytometry measuring cells within the draining lymph node of uninfected mice (N=2) and mice after infection with host-adapted Bb (N=4). Number of GC B-cells, Memory B Cells, Plasmablasts and Plasma Cells did not significantly differ between WT and B-cell CR1/2 KO. Short lived (PBs) and longer lived (MMB & PCs) immune cell populations did not change in response to CR1/2 KO.

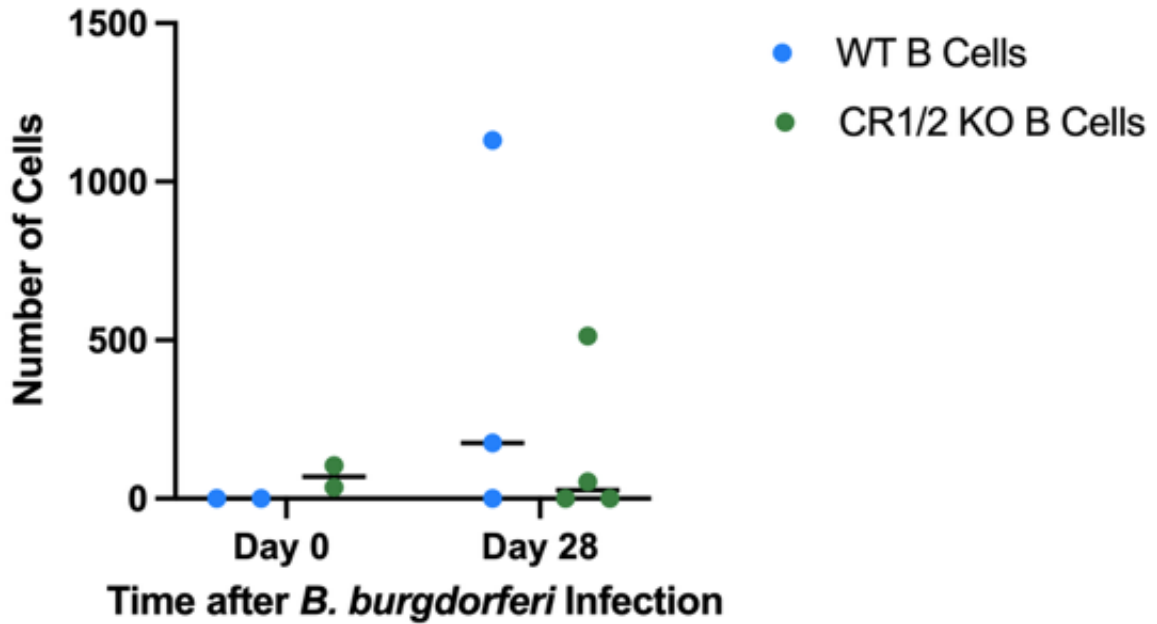


**Figure 4:** Flow cytometry measuring cells within the draining lymph node of uninfected mice (N=2) and mice after infection with host-adapted Bb (N=4). Number of T cells, activated T cells, Germinal Center Tfh cells did not differ between WT and B-Cell CR1/2 KO mice.

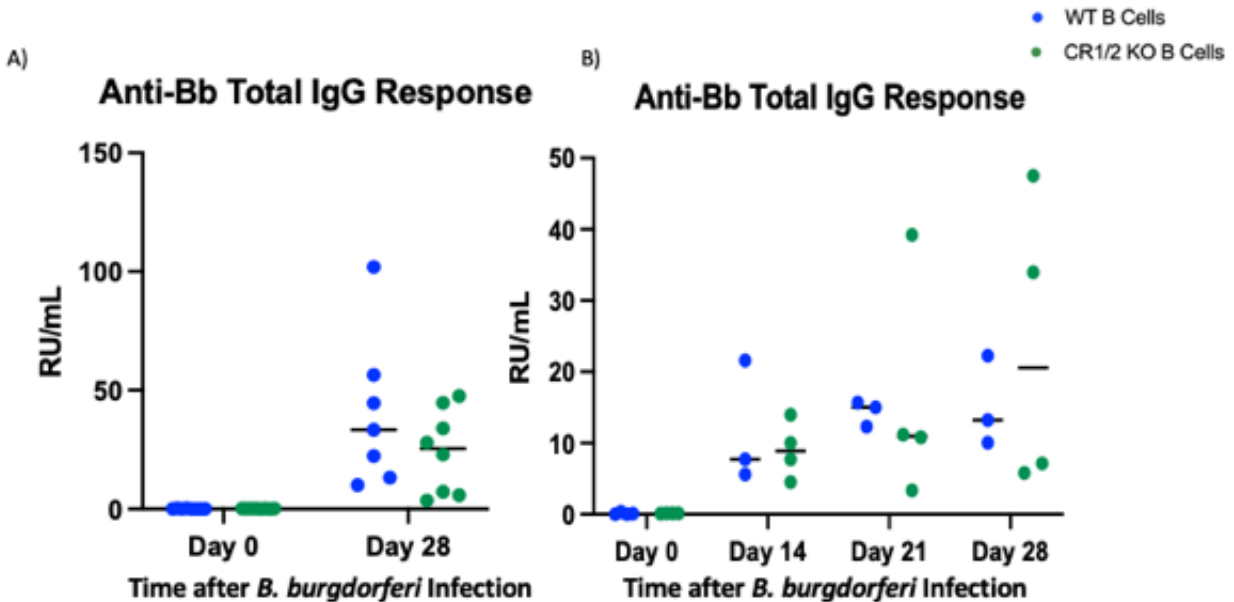


**Figure 5:** Flow cytometry measuring cells within the draining lymph node of uninfected mice (N=2) and mice after infection with host-adapted Bb (N=4). Number of FDCs did not differ between WT and B-Cell CR1/2 KO mice.

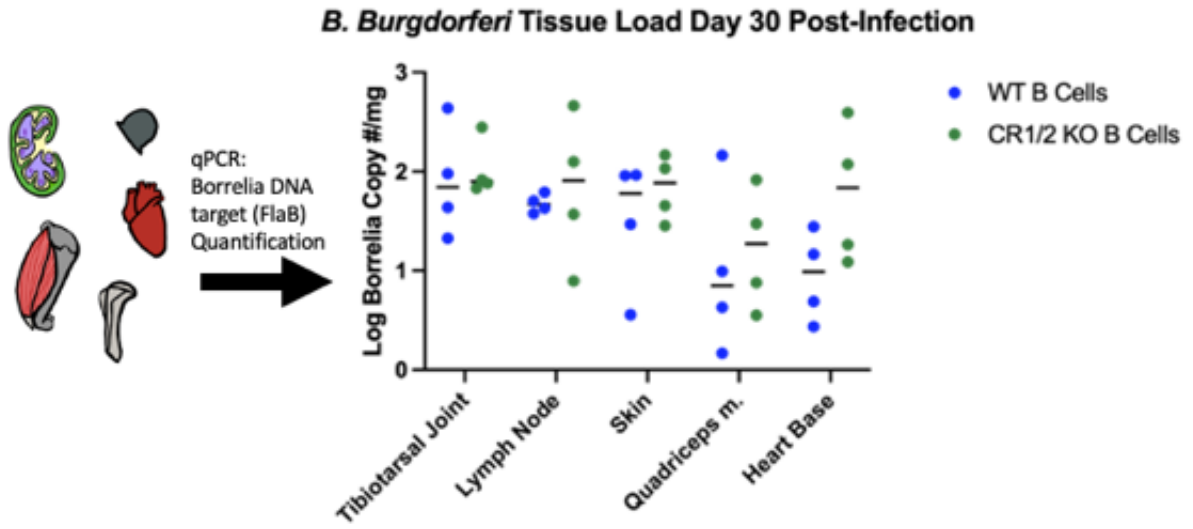
### #Follicular Dendritic Cells (FDC-M2+, ICAM+)



**Figure 6:** Serum antibody levels determined by ELISA (A) Mice were bled before infection and at day 28 after infection with host-adapted Bb (N=8) (B) Mice were bled before infection and at days 14, 21, and 28 after infection with host-adapted Bb (N=4). Anti-Bb IgG responses did not differ between WT and CR1/2 KO. Host did not produce more *B. burgdorferi* specific antibodies in response to bacterial infection.



**Figure 7:** Tissue samples from inguinal lymph node, heart base, ear skin, tibiotarsal joint, and quadriceps muscle were collected from groups of 4 mice. Bacterial burden within those tissues was measured using qPCR. Day 30 Bb Tissue Loads did not differ between WT and CR1/2 KO. Host ability to clear bacterial infection was not altered.





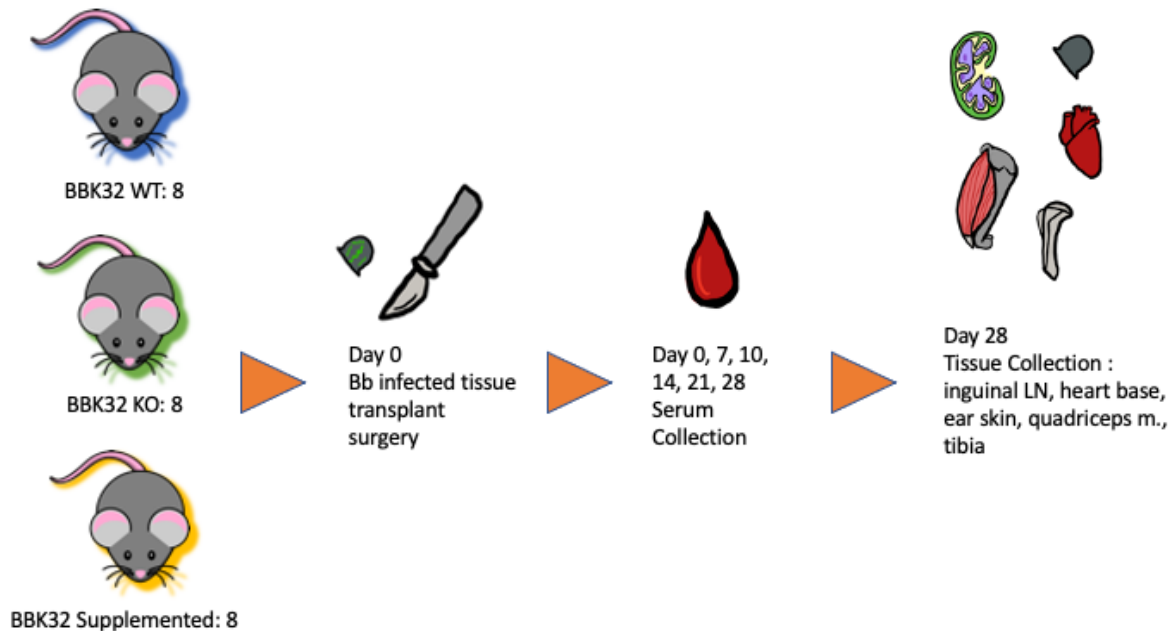
## BBK32 AND AIM 2 RESULTS

### Methods

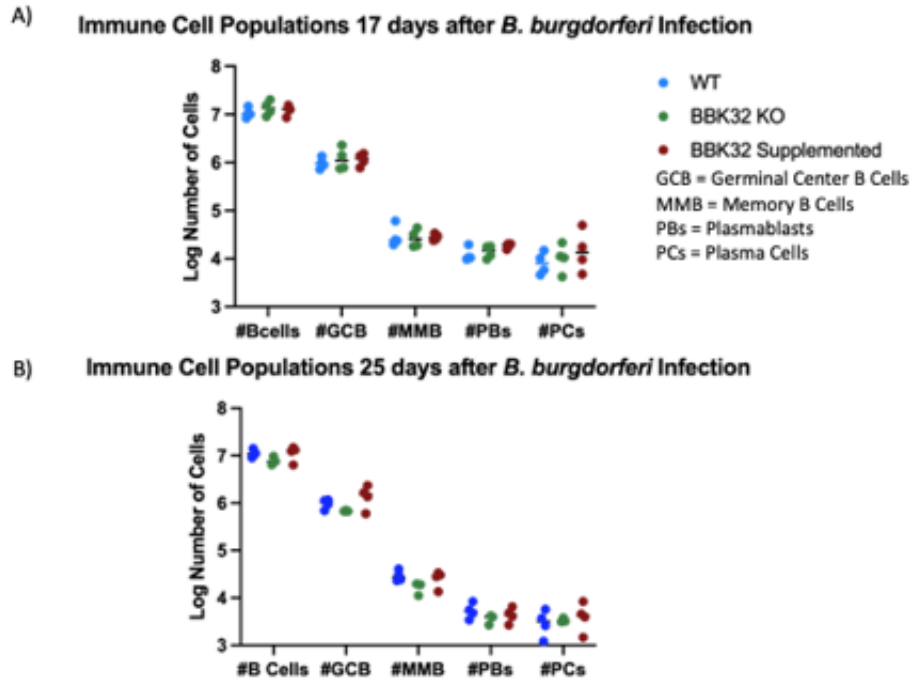
- Flow cytometry to quantify immune cell populations at day 28 post infection in the draining lymph node
- qPCR to measure *B. burgdorferi* tissue load
- ELISA serum analysis to measure anti-Bb IgG responses

**Aim 2:** Study the effects of BBK32-deficiency on B cell responses to Bb

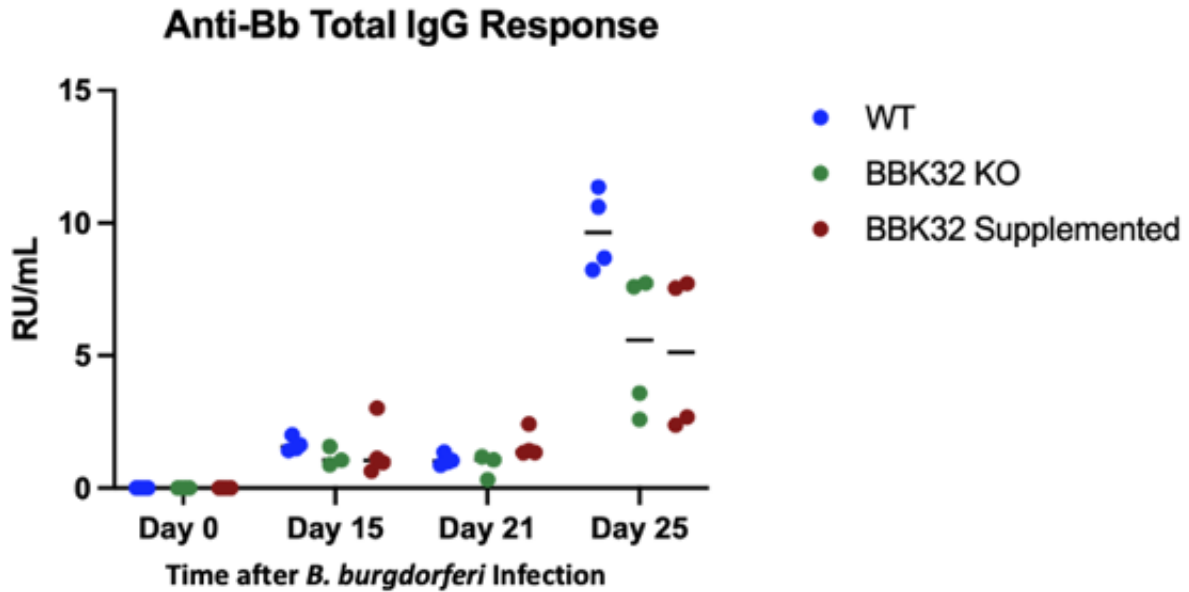
- *In vivo* experiments using WT mice infected with:
  - WT *B. burgdorferi*
  - BBK32 KO *B. burgdorferi*
  - BBK32 Supplemented *B. burgdorferi*



**Figure 8:** Flow cytometry measuring cells within the draining lymph node of mice after infection with host-adapted Bb (N=4). Number of Germinal Center B cells, Memory B Cells, Plasmablasts and Plasma Cells did not differ between WT, BBK32 KO and BBK32 Supplemented groups at Day 17 of infection (A) or Day 25 of infection (B)



**Figure 9:** Groups of 4 mice were bled before infection and at days 15, 21, and 25 after infection with host-adapted Bb. Serum antibody levels were determined by ELISA. Anti-Bb IgG responses did not differ between WT, BBK32 KO, and BBK32 Supplemented groups. Host did not produce more Bb specific antibodies in response to infection.

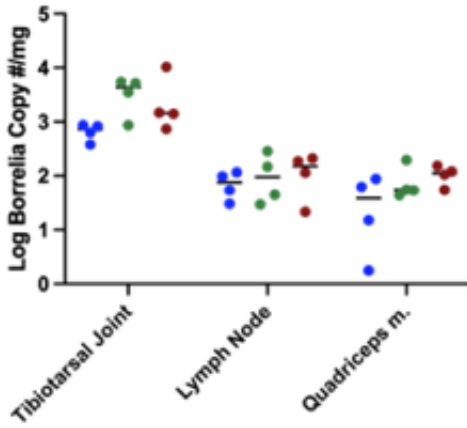


**Figure 10:** Tissue samples from inguinal lymph node, heart base, tibiotarsal joint, and quadriceps muscle were collected from groups of 4 mice. Bacterial burden within those tissues was measured using qPCR. Day 17 (A) and Day 25 (B) Bb Tissue Loads did not differ between WT, BBK32 KO, and BBK32 Supplemented groups. Host ability to clear bacterial

infection was not altered.

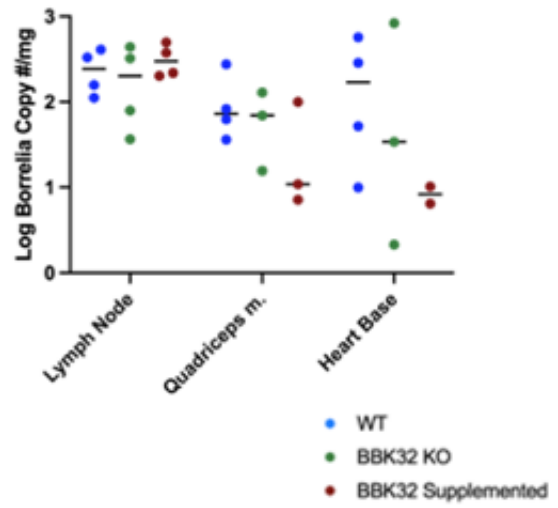
A)

**B. Burgdorferi Tissue Load Day 17 Post-Infection**



B)

**B. Burgdorferi Tissue Load Day 25 Post-Infection**



## DISCUSSION

- Surprising that knocking out a receptor important for antigen presentation shows no effect. Results could suggest that:
  - CR1/2 interference may not be an immune evasion strategy utilized by Bb.
  - **OR** complement may be so strongly inhibited by Bb that no change would be observed.
- By studying immune evasion strategies we expect to help the development of future therapeutic interventions that support pathogen clearance.
  - Future studies can attempt to overexpress complement proteins and potentially overcome Bb mediated complement inhibition.

### Acknowledgements

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