**Borrelia burgdorferi** Infection Affects Local and Systemic Complement C4 Levels in Wild Type Mice

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**INTRODUCTION**

- The spirochete *Borrelia burgdorferi* (Bb) causes Lyme Disease, the most common arthropod-borne illness in the United States¹.
- Mice infected with Bb show a strong initial B cell response, but the abnormally-structured lymph node (LN) germinal centers collapse by day 30 post-infection, obviating systemic immunity²,³.
- Germinal center (GC) failure may be due to complement-mediated B cell activation through two pathways⁴,⁵.

**Hypothesis:**

Complement C4 anchors Bb antigen (Ag) binds directly to B cell surface receptors to activate it; complement C3dg binds CR1/CR2 receptors and provides co-stimulatory signals to enhance the B cell response.

**RESULTS**

1. **Inguinal lymph node** weight of infected mice was significantly greater than that of immunized individuals at all time points (p < 0.005; two-tailed t-test, CI=95%), but declined significantly over time (p=0.0015; one-way ANOVA, CI=95%), whereas the ILN mass of the immunized group remained constant (p=0.1994; one-way ANOVA).

**METHODS**

- 36 female C57BL/6 Mice (Jackson)
- Pre-treatment blood sample
- Inguinal lymph node homogenized in Cell/Tissue Extraction Buffer + Protease Inhibitor Cocktail
- Post-treatment blood sample
- Serum and lymph node C4 quantified via sandwich ELISA (antibodies from Cedarlane)

**RESULTS**

1. **Average Draining Lymph Node Weight**
   - **Day 15**
   - **Day 21**
   - **Day 28**

**REFERENCES**


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**KEY FINDINGS**

- ELISA and immunohistochemical data suggest a significant depletion of complement C4 in the draining lymph node of infected mice at the time of GC induction (Day 15); this difference was not seen at other time points.
- Serum C4 levels showed a trend towards reduction in infected compared to immunized mice; however, the difference did not reach statistical significance.
- Draining lymph node of infected mice weighed significantly more than those of immunized mice at all time points, but the mass decreased significantly over the course of infection.

**CONCLUSIONS**

- Infection with *Borrelia burgdorferi* affects complement C4 deposition on FDCs, and overall C4 levels are reduced at some time points. Further work is required to determine whether these observed reductions are linked mechanistically to the lack of functional germinal center development after Bb infection.
- It is unlikely that the systemic increases in C4 are due to local production, since serum C4 levels continue to rise during infection, even after lymph node levels drop.
- Follow-up is needed to determine the functional effects of complement depletion on B cell activation.

**FUTURE STUDIES**

- Repeat study with a greater sample size and include a cohort of naive mice at each time point to collect baseline C4 readings.
- Transfer E.coli (Millipore) with custom-designed plasmid (GenScript) containing gene for mouse C3dg and Bir A gene for in vitro biotinylation of recombinant protein (see below).
- Apply recombinant C3dg tetramers to infected and immunized mice at Day 21 and analyze B cell levels with flow cytometry to assess whether provision of functional complement signaling sustains B cell response – potential therapeutic target.