The role of NLRP3 inflammasome in Lyme arthritis

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Background

It is estimated that there are 300,000 new cases of Lyme disease in the U.S per year.

Lyme disease is the most reported vector-borne illness in the US.

Experimental Lyme arthritis is a valuable mouse model to study the development of inflammation in C3H mice infected with *Borrelia burgdorferi*. We are interested in the role of the inflammasome in initiating inflammation due to infection of *B. burgdorferi*. We expect infection with *B. burgdorferi* will activate the non-canonical NLRP3 pathway.

NOD-like receptors (NLRs) are expressed in the cell cytosol and are able to detect intracellular pathogens. They are partly responsible for immune activation leading to production of inflammatory cytokines and inflammation. NLRP3 is the most versatile NLR and likely the most clinically important. Involvement of the NLRP3 inflammasome during infection with *B. burgdorferi* is controversial.

Project Goal**:** Define the activation of the NLRP3 inflammasome in the immune response to *Borrelia burgdorferi* infection.

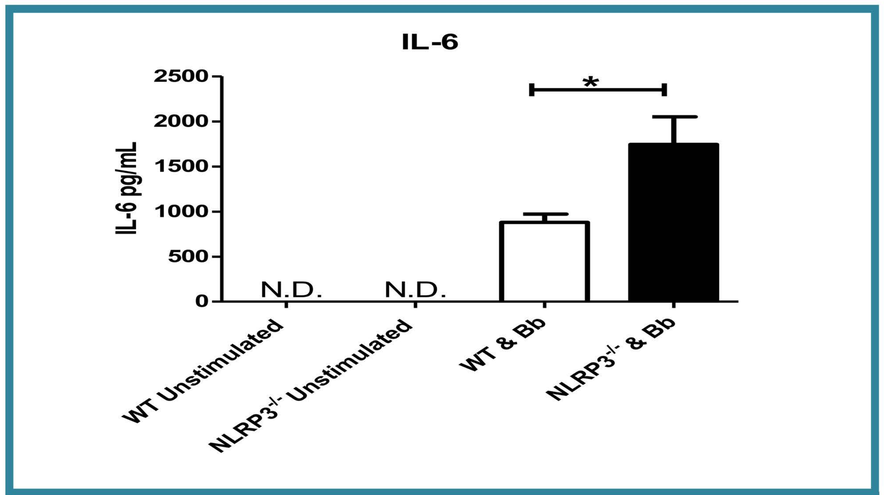


Fig 1. IL-6 levels of C57BL/6 thioglycollate-elicited peritoneal macrophages co-cultured with Bb. 24 hours. IL-6 levels were measured via ELISA. IL-6 levels are independent from inflammasome activation. *= p< 0.001

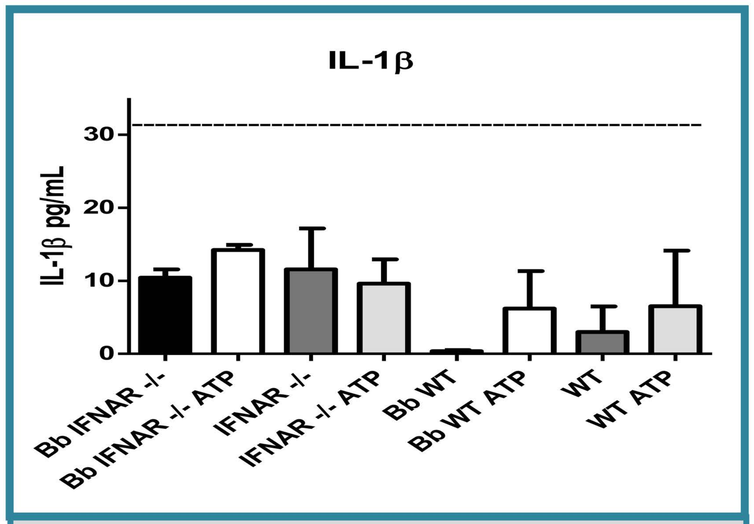


Fig 3. IL-1β levels of C3H BMDM co-cultured with Bb for 24 hours. IL 1β levels were measured via ELISA. IL-1β levels are dependent of inflammasome activation. Dotted line is the lowest detectable value.

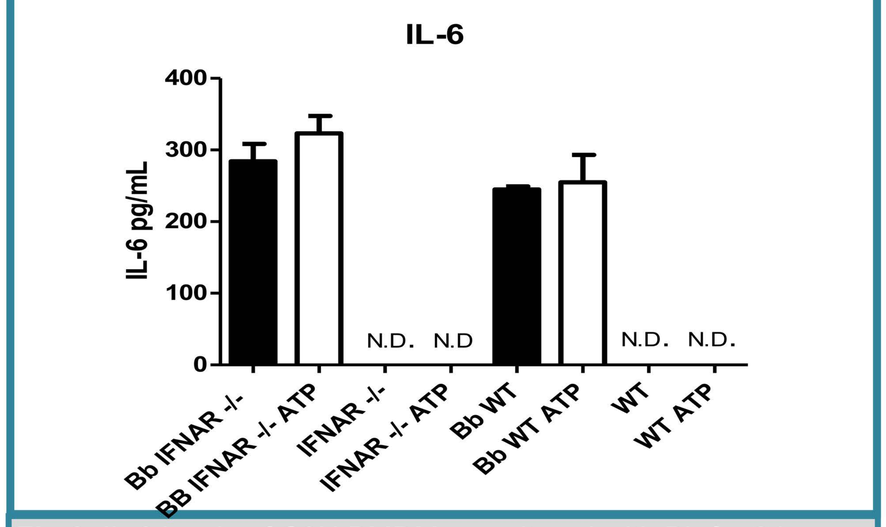


Fig 2. IL-6 levels of C3H BMDM co-cultured with Bb for 24 hours. IL-6 levels were measured via ELISA. IL-6 levels are independent from inflammasome activation.

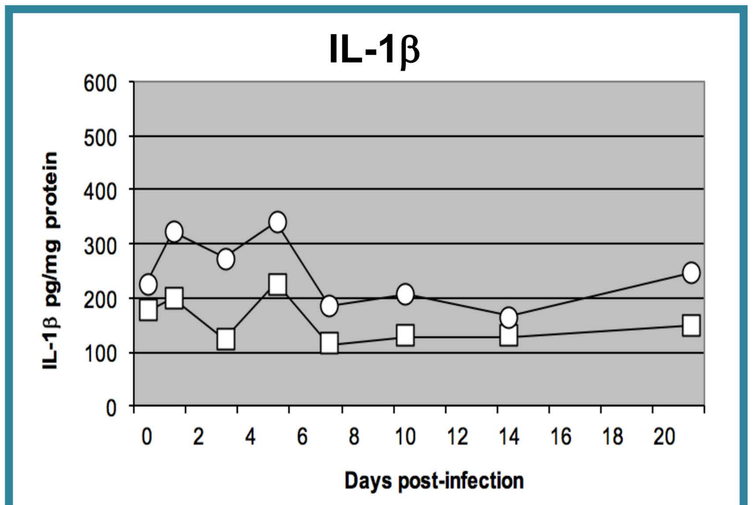
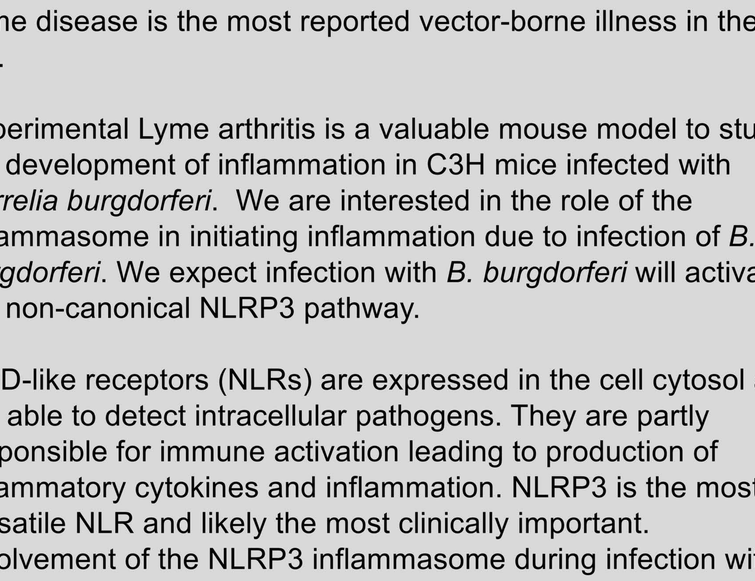


Fig 4. Cytokine levels of infected ankle joints at various times post infection with Bb. Circles are C3H mice and squares are C57BL/6 mice.



This is our protocol for isolating and stimulating bone marrow derived macrophages.

Methods

Bone marrow derived macrophages
Harvest BMDM were plated in complete DMEM with 30% L929 supernatant for 5 days, allowing them to differentiate and adhere.
Resident peritoneal macrophages
Resident peritoneal macrophages (rPM) from C3H mice were harvested by washing the peritoneum with sterile PBS. rPM were plated in complete RPMI media for 24 hours.
In vitro :
C3H BMDM were cultured and stimulated with *Borrelia burgdorferi (Bb)* strain NP40 at MOI of 10 and left for 24 hours. Cell supernatant was harvested and used to analyze production of IL-1β and IL-6 by ELISA.
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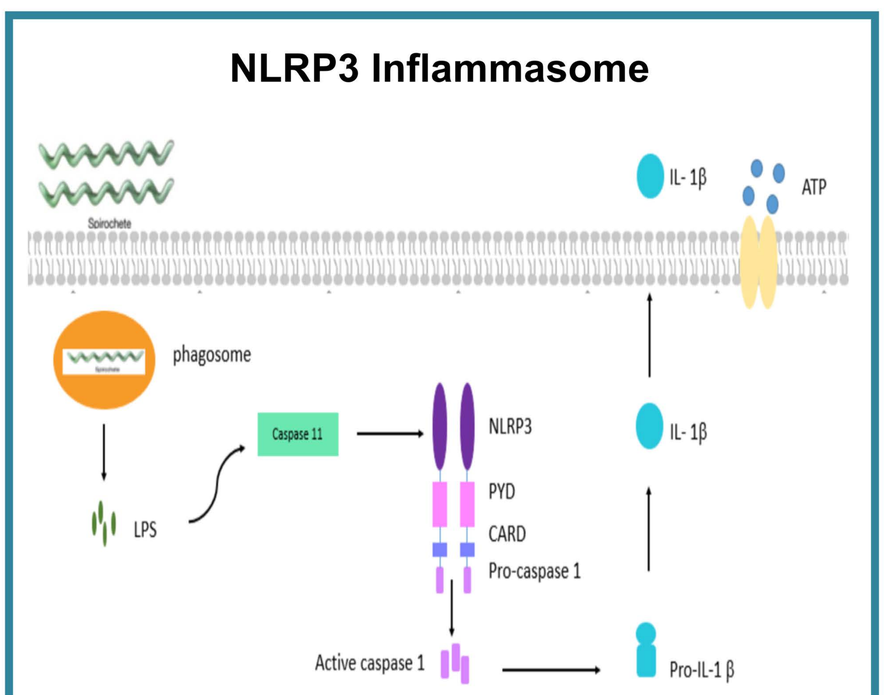
In vivo: C3H and B6 WT mice were infected with 50 µl of 2.5 x 10^5 *Bb* in each hind footpad.



Toll-like receptors recognize LPS leading to a signaling cascade. Autocrine signaling of IFNAR activates caspase 11, which then actives the NLRP3 inflammasome. NLRP3 activates caspase 1 which then cleaves pro-IL-1β to its active form, IL-1β. We are investigating the role of the NLRP3 inflammasome in Lyme arthritis. IL-6 is independent of inflammasome activation.

Results

We did not see any detectable values of IL-1β in our our ELISA's. We use an MOI of 10 and will increase it to see if we get inflammasome activation. C3H mice produce more cytokines when infected with Bb compared to C57BL/6 mice.

Future Directions

For future experiments we will be using an NLRP3 inhibitor in an in vivo experiment. Investigate the role of the NLRP3 inflammasome in C57BL/6 mice compared to C3H mice.

We will redo our experiment and stimulate with a higher dose of Bb.

Acknowledgements

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