Role of COX-2 and 5-LO in mediating macrophage inflammatory response to *Borrelia burgdorferi*

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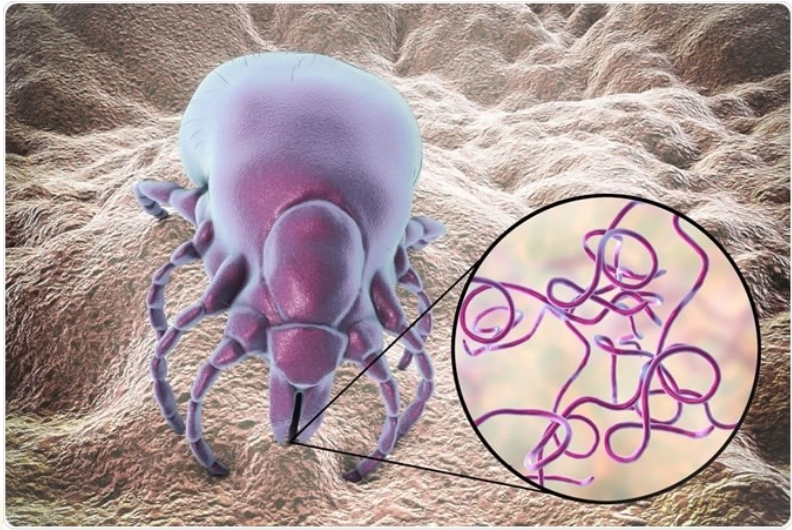
Background:

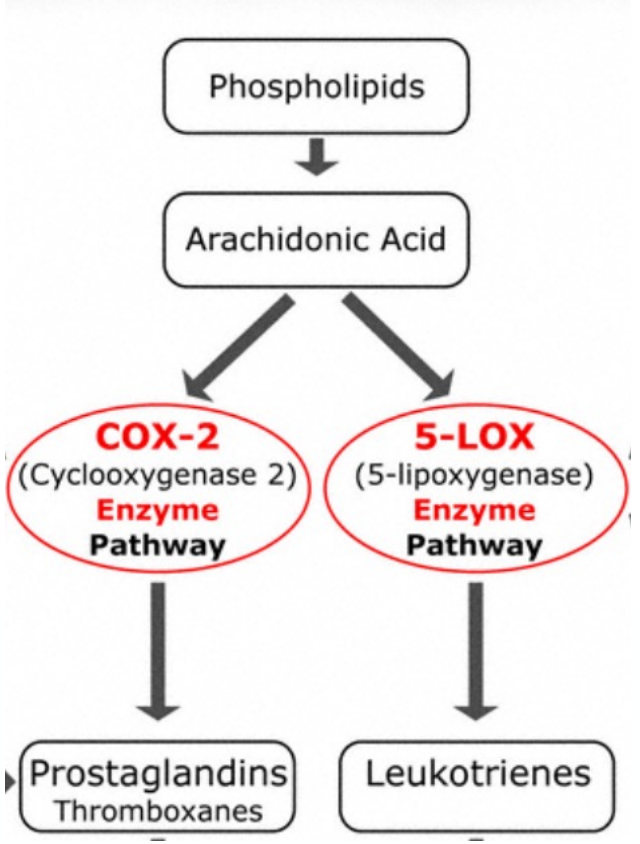
- Lyme Disease is the most common tick-borne disease in the United States.
- *Borrelia burgdorferi (Bb)* is the spirochete that causes the disease and is transferred through a tick bite.
- Infection pathogenesis creates an inflammatory response and that can develop into arthritis and other complications.



https://www.news-medical.net/news/20191218/New-paper-based-test for-early-Lyme-diagnosis.aspx

Background:

- Macrophages and neutrophils mediate the initial response to the infection.
- Arachidonic acid (AA), a component of the cellular membrane, is released during infection and enzymes will metabolize AA into eicosanoids that mediate inflammation.
- 5-lipoxgenase (5-LO) and cyclooxygenase-2 (COX-2) are the initial enzymes that metabolize AA into different classes of eicosanoids.



Hypothesis:

We hypothesize that 5-LO and COX-2 are important components of the initial immune response and production of pro-inflammatory cytokines by macrophages co-cultured with *B. burgdorferi.*

Approach:

To test this hypothesis, RAW 264.7 macrophages were pre-treated with MK-886 and/or CAY10404 to block the activity of 5-LO and/or COX-2. After 24h co-culture with *B. burgdorferi,* production of the pro-inflammatory cytokine TNF-α and anti-inflammatory cytokine IL-10 were examined.

Methods/Techniques:

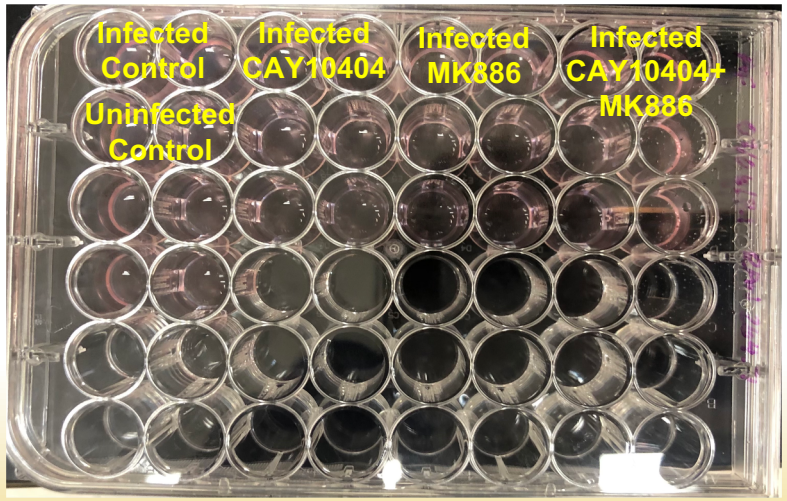
- Grow and maintain RAW 267.4 macrophages & *Bb* cultures.
- Count RAW 267.4 macrophages & *Bb* in microscope and calculate number of cells or *Bb* to seed wells.
- Perform different ELISA protocols and interpret results.

Experiments:

Day 1: Plate RAW 264.7 macrophages onto a 48-well plate, let them attach overnight.

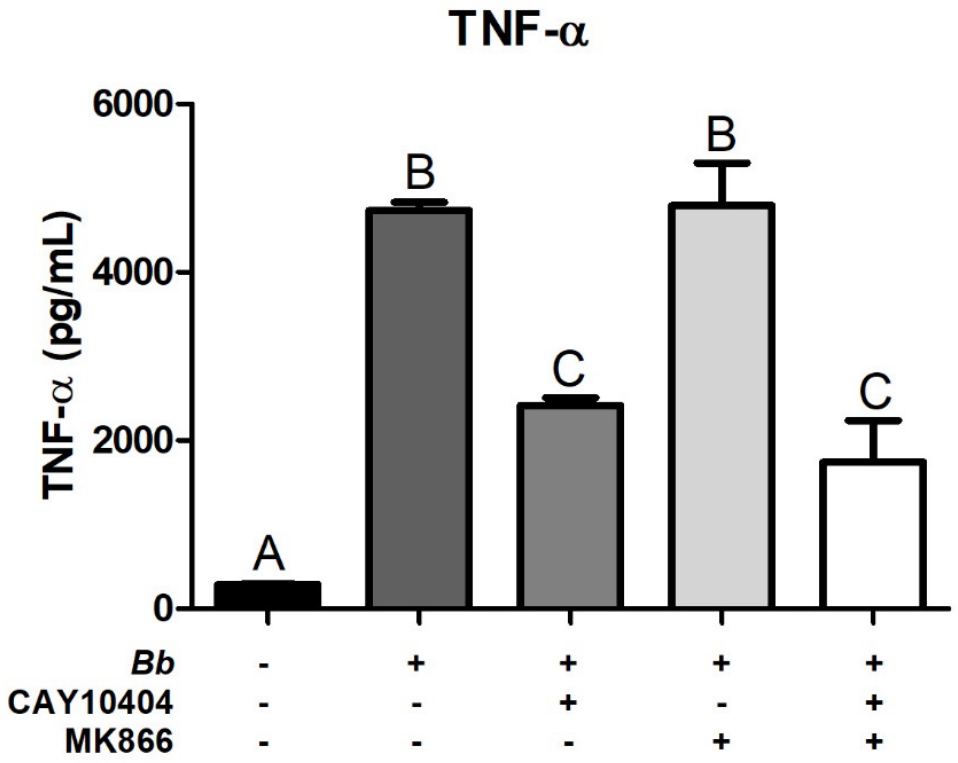
Day 2: Prime cultures for ten minutes with MK-886 and/or CAY10404 (10μM) and infect with *Bb* (MOI 10).

Day 3: Collect supernatants after 24h and measure TNF-α and IL-10 production using ELISA.

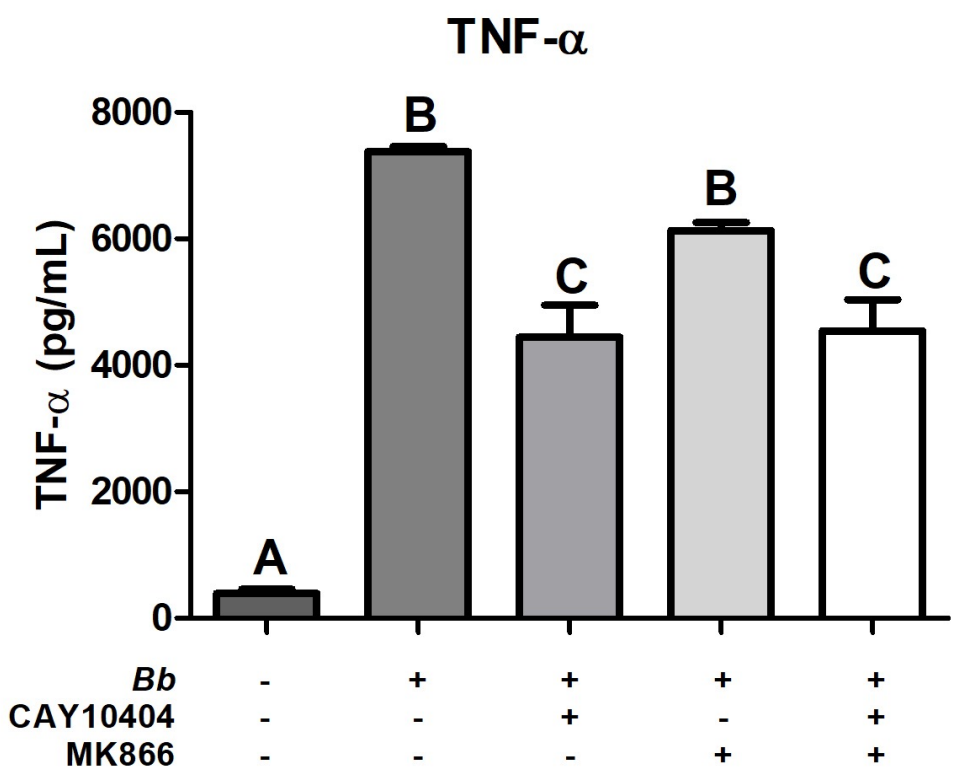


ELISA Data:

Trial 1



Trial 2



*Working on running IL-10 ELISAs on samples

- Bars with different letters are significantly different from others at $P < 0.5$ level by 1-way ANOVA with Tukey's post-test

Conclusion:

- From these results, we see that blocking the COX-2, but not the 5-LO pathway, significantly impacted TNF-α production by RAW 264.7 macrophages co-cultured with *Borrelia burgdorferi.*
- The effect of the CAY10404 treatment shows that the eicosanoids derived from the COX-2 pathway play a larger role, compared to 5-LO, in stimulating the cytokine response in macrophages co-cultured *B. burgdorferi* i*n vitro* 24h

Future Direction:

- Future studies will focus on the role of different eicosanoids in inflammatory response of macrophages to *Borrelia burgdorferi*
  - Determine how varying dosages of CAY10404 will affect macrophage cytokine response to co-cultured with *Borrelia burgdorferi* when pretreated with varying dosages of CAY10404 (COX-2 inhibitor).
  - Determine how activating the 5-LO pathway will affect macrophage cytokine production

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