Role of peroxisome proliferator-activated receptor γ (PPARγ) in *Coxiella burnetii* infection

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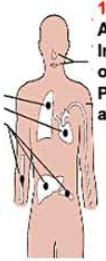
Introduction

Coxiella burnetii

- Gram negative bacterium
- Causative agent of human Q fever



1. Entry
Aerosol transmission
Infectious dose (< 10 organisms)
Potential bio-terror agent

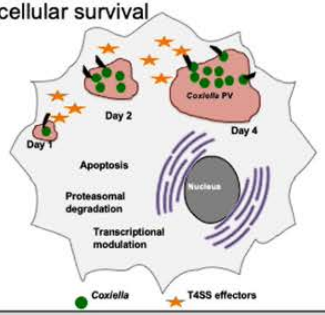
2. Spread
Hematogenous (through blood)

3. Disease
Acute
– Pneumonitis
Chronic
– Endocarditis
– Granulomas

4. Exit
Usually none in man

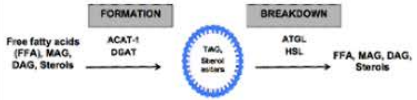
Pathogenesis of *Coxiella*

- Preferably infects alveolar macrophages
- Found in lipid droplet-rich foam cells in endocarditis patients
- Parasitophorous vacuole (PV) is essential for bacterial growth
- Uses Type 4 Secretion System (T4SS) to manipulate host cells
- Lipid droplets are important for *Coxiella* intracellular survival



Lipid droplets are storage organelles important for cellular metabolism

- Neutral lipid storage organelles
- Store esterified cholesterol and free fatty acids (triacyglycerol)
- Biogenesis from ER
- Functions - Energy homeostasis, membrane trafficking, signaling

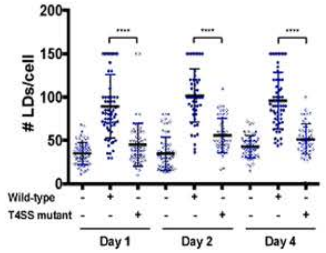


MAG, DAG, TAG– Mono, Di and Triacylglycerol, DGAT – Diacyl glycerol acyl transferase, ACAT-1 – Acyl coenzyme A acetyl transferase, ATGL – Adipose triglyceride lipase , HSL – Hormone sensitive lipase

Preliminary Data

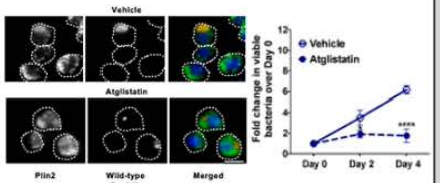
Are lipid droplets important for *Coxiella* intracellular pathogenesis?

Figure 1: Lipid droplet accumulation is dependent on the *Coxiella* T4SS



Wild-type and T4SS mutant *Coxiella*-infected mouse alveolar macrophages (MH-S) cells were stained for Plin2 and *Coxiella*. Number of lipid droplets were counted by fluorescence microscopy. Graph represents number of lipid droplets/cell in uninfected and infected cells. (n=3) ****=p<0.0001 determined by two-way ANOVA.

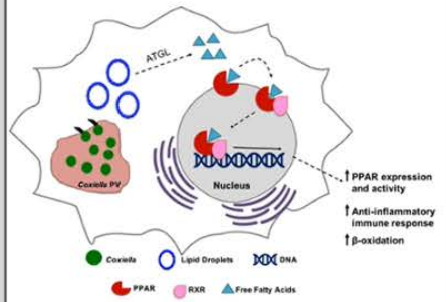
Figure 2: Blocking lipid droplet breakdown results in reduced *Coxiella* growth



MH-S cells infected with wild-type *Coxiella* were treated with vehicle (DMSO) and 20uM atglistatin. Bacterial growth was determined at day 4 by FFU Assay (n=3) *=p<0.05,**** =p<0.0001 compared to vehicle-treated cells two-way ANOVA with Bonferroni post-hoc test. Scale bar = 10 μm.

Lipid droplets and PPARγ

- Lipid droplet breakdown releases free fatty acids (FFAs)
- FFAs are PPARγ agonists
- Activation of PPARγ induces anti-inflammatory immune response
- Example: *Mycobacterium tuberculosis, Mycobacterium leprae*



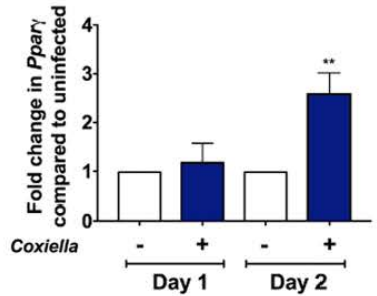
Coxiella breaks down lipid droplets in presence of the enzyme ATGL to release Free Fatty Acids (FFAs) which act as PPARγ agonists. The activated PPARγ receptor then heterodimerizes with Retinoid X Receptor (RXR) and translocates to the nucleus, binds to PPAR response elements (PPRE) and regulates expression of several genes influencing cellular β-oxidation, host immune response etc.

Overall Question

Does *Coxiella* infection affect PPARγ expression and activity to induce anti-inflammatory immune response?

Results

Figure 3: *Coxiella* infection upregulates PPARγ expression in infected alveolar macrophages



MH-S cells were infected with WT *Coxiella*. RNA was collected Day 1 and 2 post-infection, reverse transcribed to cDNA and gene expression was determined using quantitative Real Time (qRT-PCR). Fold change was calculated compared to uninfected samples using GAPDH expression as housekeeping. **=p<0.01 as determined by One-way ANOVA with Tukeys post-hoc test

Conclusions

Coxiella infection upregulates *PPARγ* gene expression in alveolar macrophages

- suggests *Coxiella* might manipulate *PPARγ* expression and activity to induce an anti-inflammatory immune response to promote intracellular survival.