Contribution of lipid droplet breakdown during *Coxiella burnetii* infection

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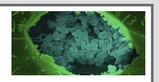
Abstract

Coxiella burnetii **is an obligate intracellular bacterium and causative agent of culture-negative endocarditis. Although *Coxiella* initially infects alveolar macrophages, it is found in lipid droplet (LD)-containing foamy macrophages in endocarditis patients. LDs are host lipid storage organelles containing cholesterol esters (CE) and triacylglycerols (TAG). Our previous studies show that *Coxiella* actively manipulates host LD metabolism via its Type 4 Secretion System (T4SS), which secretes bacterial effectors in the host cell cytoplasm to manipulate cellular processes. Further, specifically blocking adipose triglyceride lipase (ATGL)-mediated LD breakdown inhibits *Coxiella* growth suggesting importance of LD-derived lipids for bacterial growth. However, how *Coxiella* regulates LD breakdown and the composition of LD-derived lipids is unknown. Our preliminary fluorescence microscopy studies using CRISPR knockouts and LD inhibitors indicate presence of TAG-rich LDs in *Coxiella*-infected cells. ATGL-mediated breakdown of TAG-rich LDs releases arachidonic acids, precursors for lipid immune mediators important for immunomodulation during bacterial infections. Hence we hypothesize that *Coxiella* manipulates *ATGL via its T4SS to initiate TAG-rich LD breakdown and subsequently modulate the immune response to promote bacterial survival.* To test this hypothesis, we analyzed ATGL gene expression in differentially infected cells using qRT-PCR. Compared to uninfected and T4SS-infected cells, *Coxiella* infection increased ATGL expression indicating T4SS-dependent regulation of ATGL. Ongoing studies are elucidating the *Coxiella* T4SS-ATGL interaction. To identify cellular CE and TAG levels and the breakdown products at different times post-infection, we are performing thin layer chromatography (TLC). Completion of our studies will identify the LD breakdown-derived lipids and how *Coxiella* regulates LD breakdown of to promote its intracellular survival.**

Introduction

Coxiella burnetii



- **Gram negative bacterium**
- **Causative agent of human Q fever**

3. Disease
Acute
– Pneumonitis
Chronic
– Endocarditis
– Granulomas

4. Exit
Usually none in man

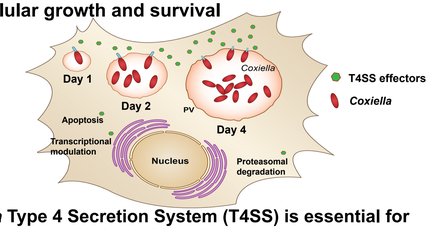
1. Entry
Aerosol transmission
Infectious dose (< 10 organisms)
Potential bio-terror agent

2. Spread
Hematogenous (through blood)

Ref: http://microwikiwau.wikispaces.com/

Coxiella is an obligate intracellular pathogen

- ***Coxiella*** **preferentially infects alveolar macrophages**
- **The parasitophorous vacuole (PV) is central to *Coxiella* intracellular growth and survival**



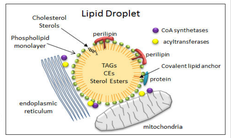
- ***Coxiella*** **Type 4 Secretion System (T4SS) is essential for intracellular survival and PV maintenance**
- **Host lipids play an important role in *Coxiella* pathogenesis**

Lipid Droplets and *Coxiella*

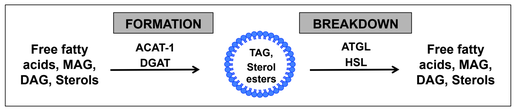
- ***Coxiella*** **is found in lipid droplet-rich foamy macrophages** *(Broquoi et al, 1994)*
- **Two separate microarray analyses reported differential regulation of the lipid droplet coat protein *plin-2* in *Coxiella*-infected human macrophage-like cells (THP-1)** *(Ron et al2003, Mahapatra et al 2010)*
- **Lipid droplets were observed in the *Coxiella* PV lumen of infected human alveolar macrophages** *(Graham et al, 2013)*
- **siRNA depletion of ATGL, the phospholipase involved in LD breakdown, increased the number of *Coxiella* PVs in HeLa epithelial cells** *(McDonough et al 2013)*
- **Treatment of Vero cells with a broad spectrum antiviral molecule ST699 which localizes to host cell lipid droplets inhibited *Coxiella* intracellular growth** *(Sandoz et al 2014)*

Lipid droplets are host lipid storage organelles

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



Lipid droplet homeostasis

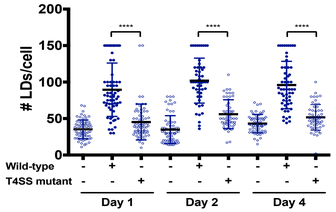


MAG, DAG, TAG– Mono, Di and Triacylglycerol, DGAT – Diacyl glycerol acyl transferase, ACAT-1 – Acyl coenzyme A acetyl transferase, ATGL – Adipose triglyceride lipase , HSL – Hormone sensitive lipase

Preliminary Data

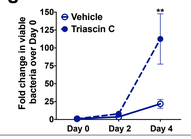
Are lipid droplets important during *Coxiella* intracellular pathogenesis?

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



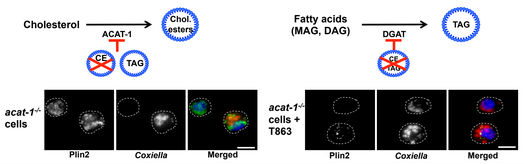
Wild-type and T4SS mutant *Coxiella*-infected cells were stained for Plin2 and *Coxiella*. Number of lipid droplets were counted by fluorescence microscopy. Graph represents number of lipid droplets/cell in uninfected and infected cells. (n=3)

Figure 2: Blocking lipid droplet formation increases *Coxiella* growth



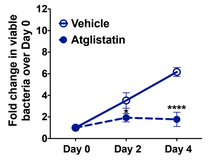
Wild-type *Coxiella*-infected cells were treated with vehicle (DMSO) and 20uM Triacsin C. Bacterial growth was measured by FFU Assay (n=3) **= p <0.01, compared to vehicle-treated cells two-way ANOVA with Bonferroni post-hoc test.

Figure 3: Lipid droplets in *Coxiella*-infected cells appear TAG-rich



Wild-type *Coxiella*-infected *acat-1 -/-* cells were treated with vehicle (DMSO) and 20uMT863 (DGAT inhibitor). Cells were stained for Plin2 and *Coxiella* 4 days post-infection and visualized under a Leica fluorescence microscope (100X). Scale bar = 10um

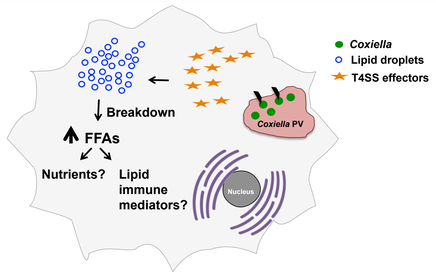
Figure 4: Blocking lipid droplet breakdown results in reduced *Coxiella* growth



Cells infected with wild-type *Coxiella* were treated with vehicle (DMSO) and 20uM atglistatin. Bacterial growth was measured by FFU Assay (n=3) **= p <0.01, **** =p<0.0001 compared to vehicle-treated two-way ANOVA with Bonferroni post-hoc test.

Overall Goal

How do lipid droplets contribute to *Coxiella* intracellular growth?



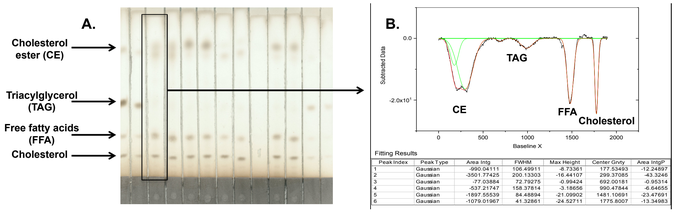
1. **What is the composition of lipid droplets at different times post-infection?**
2. **How does *Coxiella* regulate lipid droplet breakdown?**

Methods and Results

Question 1: What is the composition of lipid droplets?

Figure 5: Quantification of lipids in *Coxiella*-infected cells

Extract Lipids using chloroform-methanol and prepare different standard concentrations
↓
Load the samples on silica glass plate
↓
Separate lipids in the solvent mixture: hexane/diethyl ether/acetic acid
↓
Develop the TLC plates
↓
Analyze plate picture using ImageJ, followed by OriginPro
↓
Based on the obtained standard, quantify the lipid concentration
↓
Normalize the lipid concentration to protein concentration



Fitting Results

Peak Index	Peak Type	Area Intg	FWHM	Max Height	Center Grvty	Area Intg%
1	Gaussian	-660.04111	106.49811	-8.73361	177.53493	-12.24997
2	Gaussian	-3501.17425	200.13303	-16.44107	299.37085	-43.3246
3	Gaussian	-77.03884	72.79275	-0.99424	692.00181	-0.95314
4	Gaussian	-537.21747	158.37814	-3.18656	990.47844	-6.64655
5	Gaussian	-1937.55539	84.48894	-21.09902	1481.10691	-23.47691
6	Gaussian	-1079.01967	41.32861	-24.52711	1775.8007	-13.34983

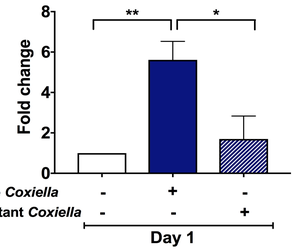
Lipids extracted from uninfected and wild-type *Coxiella*-infected cells, and lipid standards were separated by TLC. A. Developed TLC plate and, B. Quantification of lipids performed in OriginPro indicating particular lipid concentration. This is still work in progress.

Question 2: How does *Coxiella* regulate lipid droplets?

ATGL expression using Quantitative PCR (qPCR)

Alveolar macrophages infected with *Coxiella*
↓
RNA extracted using Qiagen Rneasy mini kit
↓
RNA reverse transcribed to cDNA
↓
ATGL gene expression using quantitative Real-Time PCR

Figure 6: Upregulation of ATGL expression is *Coxiella* T4SS-dependent



RNA was collected from wild-type and T4SS mutant *Coxiella* infected MH-S cells at day 1 post-infection. Gene expression analysis was performed using standard quantitative RT PCR. Fold change was calculated compared to uninfected samples. One-way ANOVA was used for statistical analysis (n=2) (***p < 0.001)

Conclusions

1. **ATGL is upregulated in *Coxiella* T4SS dependent manner.**
   - **Suggests manipulation of ATGL-mediated lipid droplet breakdown.**

Ongoing Studies

1. **Quantify lipid composition of wild-type and T4SS mutant *Coxiella*-infected cells at different times post-infection.**
2. **Identify the contribution of different lipid species during Coxiella infection.**
3. **Determine the regulation of ATGL at different times post-infection**

Acknowledgements

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