The Role of Lipid Droplets in Host-pathogen Interactions of Intracellular and Extracellular Bacteria

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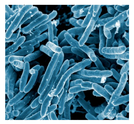
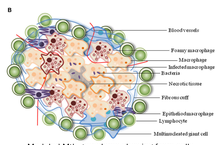
Mycobacterium tuberculosis

- Causative agent of:
  - Tuberculosis



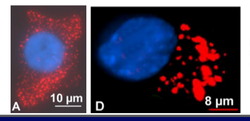
- Pathogenesis
  - Aerobic intracellular pathogen with unusual cell wall
  - Infects alveolar macrophages
  - Evades phagocytosis and prevents phagolysosomal fusion
  - Survives long-term within granulomas
- Lipid droplets & Mtb:
  - Mtb infection increases host cell lipid droplets resulting in presence of foamy lipid-laden cells within the granuloma (Peyron et al., 2008)



Modeled Mtb granuloma showing foam cells (Guirado et al, 2013)

Importance of Lipid droplets:
- Nutrient reservoir for Mtb
- Help Mtb persist in dormancy-like state, avoid anti antimycobacterial drugs and host defense mechanisms (Daniel et al., 2011)
- Source of eicosanoids

Mechanisms used by Mtb to increase lipid droplets:
- Upregulation of TAG synthesis enzyme gene to accumulate lipid droplets
- Upregulate transcription of HIF-1 to increase IFNγ production (Knight et al., 2018)
  - IFNγ increases lipid droplet formation
- Upregulates *PPAR* γ genes
  - PPAR γ is important in lipid metabolism and influences lipid droplet formation
- Mtb also induces formation of bacterial lipid droplets



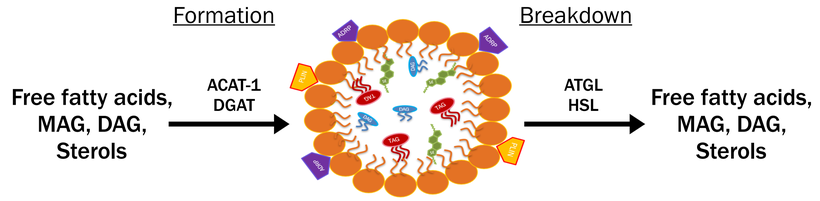
Mtb mobilizes macrophage triacylglycerol labeled with fluorescent fatty acid and accumulates fluorescent intracellular lipid droplets. (Daniel, et al.)

Goal

Summarize the published literature describing the pathways that extracellular and intracellular bacterial pathogens employ to manipulate lipid droplets and identify the contribution of lipid droplets to bacterial intracellular survival and infectivity

What are Lipid Droplets?

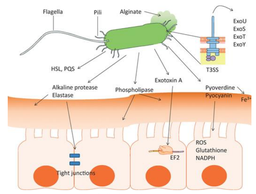
- Cytoplasmic lipid storage organelles surrounded by a phospholipid monolayer
- Store excess cellular free fatty acids and cholesterol as triacylglycerol (TAG) and cholesterol ester (CE) respectively
- Crucial to lipid metabolism and energy homeostasis
- Play a significant role in membrane trafficking, cell signaling, and inflammation which make them a prime target for pathogens



LD= Lipid droplet SE= sterol ester PLIN= Perilipin, LD- bound protein ADRP= Adipocyte Differentiation Related Protein, LD- bound protein
TAG= Triacylglycerol DAG= Diacylglycerol MAG, DAG, TAG — Mono-, Di-, and Triacylglycerol DGAT — Diacylglycerol acyl transferase
ACAT-1 — Acyl coenzyme A acetyl transferase ATGL —Adipocyte triglyceride lipase HSL — Hormone-sensitive lipase

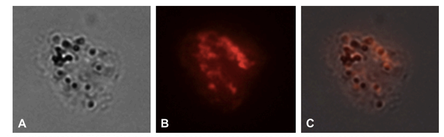
Pseudomonas aeruginosa

- Causative agent of:
  - Lung, skin, urinary tract, ear and eye infection
- Pathogenesis
  - Extracellular, obligate aerobic or facultative anaerobic bacterium
  - Opportunistic pathogen
  - Virulence factors:
    - Adhesins,
    - secreted toxins,
    - effectors secreted via the Type 3 Secretion System (T3SS),
    - antimicrobial resistance



Gellatly et al.

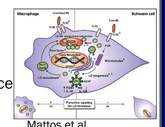
- Lipid droplets & *P. aeruginosa*
  - ExoU is a T3SS effector which exhibits phospholipase activity
  - In infected cells, *P. aeruginosa* ExoU mobilizes lipid droplets to release arachidonic acids, a source of lipid immune mediator prostaglandin.
  - Prostaglandin is important in survival of *P. aeruginosa* by increasing inflammation. (Phillips et al.)



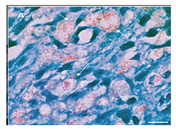
Light micrographs of PA103-infected cells at 3 h post-infection showing lipid droplets detected by phase contrast microscopy (A) and newly synthesized PGE2 detected by cell labelling with a polyclonal anti-PGE2 antibody and with a secondary antibody-Texas Red complex (B). Merged image shows localization of PGE2 in lipid bodies (C). (Phillips, et al.)

Mycobacterium leprae

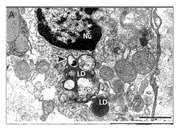
- Causative agent of:
  - Leprosy (tuberculoid, lepromatous)
- Pathogenesis
  - Obligate intracellular pathogen
  - Lepromatous: Infected Schwann cells show foam cell appearance
  - Tuberculoid: Schwann cells & macrophages form granuloma
- Lipid droplets & *M. leprae*
  - TLR2 signaling blocks expression of lipid coat protein, perilipin (Mattos, et al.)
  - Overrides TLR2-mediated blockage to induce lipid droplet formation (Mattos, et al.)
  - This partially accounts for lipid aggregation during lepromatous leprosy



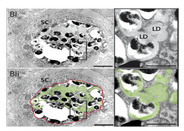
Mattos et al.



Wade staining showing foamy SCs with multiple acid–fast bacilli (arrows). (Mattos et al.)

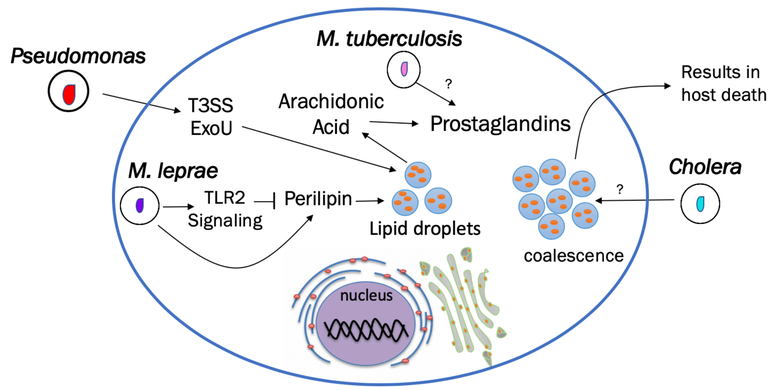


A typical LD is seen in close apposition (arrowheads) to a phagosome–containing bacterium (arrow). (Mattos et al.)



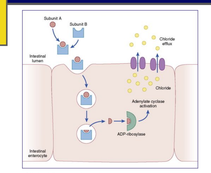
Bi. A large phagosome (outlined in red in Bii) shows a large number of bacteria enmeshed in a LD content (highlighted in green in Bii). Nu, nucleus. Bars, 1μm (A and B), 500nm (high magnification of boxed areas).

Lipid Droplet and Bacterial Interactions

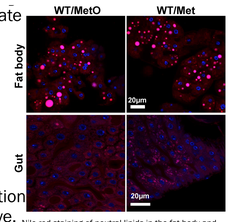


Vibrio cholerae

- Causative agent of:
  - Life-threatening diarrheal disease, particularly in developing countries
- Pathogenesis
  - Extracellular, gram-negative, facultative anaerobic pathogen
  - Cholera toxin which continually stimulates adenylate cyclase within enterocytes ⟶ profuse, watery diarrhea
- Lipid droplets & *V. Cholerae*
  - Infection results in coalescence of lipid droplets within enterocytes of small intestine in fruit flies
  - Increased lipid droplet coalescence results in depletion of lipids resulting in host death.
  - *V. cholerae* manipulates this process by degradation of metabolic methionine sulfoxide (MetO) (Vanhove, et. al)



Clinical Bacteriology, Actor



Nile red staining of neutral lipids in the fat body and intestine of flies fed LB broth or LB broth inoculated with wild-type *V. cholerae*(WT) supplemented with methionine (Met) or methionine sulfoxide (MetO) (Vanhove, et al.)

Conclusion

- *M. tuberculosis* and *Pseudomonas* promote infection in part by increasing prostaglandin production to increase inflammation
  - *Pseudomonas* exoU and T3SS increase arachidonic acid, a precursor for prostaglandins
  - *M. tuberculosis* increases prostaglandins through a poorly understood mechanism
- *M. leprae* increases LD formation and creates foam cells to increase infectivity
- *V. cholera* causes LD coalescence through a poorly understood mechanism
- Intracellular and extracellular pathogens utilize lipid droplets by varied mechanisms to promote their survival in the host.

Acknowledgements

The lipid droplet dream team: Adam McDevitt, Ahila, Cassie Libbing, Minal Mulye, Rea Azcueta