Case 3: Cruise Holiday

Bacterial Pathogenesis

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#1 - Encounter

Where does the organism normally reside?

- Freshwater environments (ie. Rivers and lakes)
- Amoeba & ciliated protozoa (Hartmanella, Acanthamoeba and Naeglaria species): Provide protection against environment & allow growth during infection
- Geographical residence: Incidence of disease in USA, Canada, New Zealand, Australia, Japan, Singapore, and Europe
- Hospitals: Nosocomial infections - more common in developed countries

Bacterial characteristics that suit Legionella for its places of residence

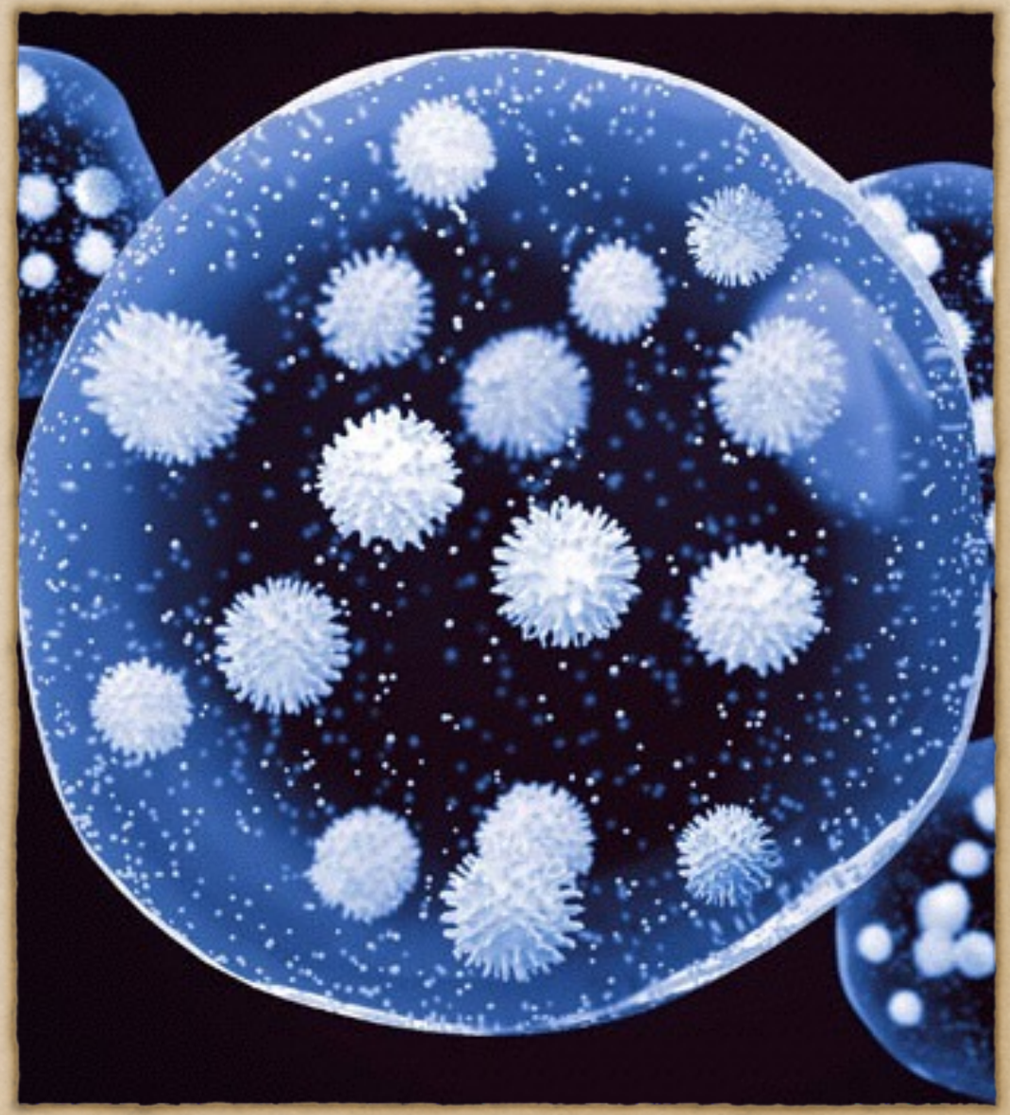
- Warm, moist soils
- Optimal growing temperature: 35 degrees celsius (like in hot tubs and pools)
    - Generally can grow between 20-50 degrees celsius
- Aerosols and droplets
- Contaminated drinking water entering respiratory tract through inhalation/swallowing

Hot Tubs & RWI (Recreational Water Illnesses)

- High temperatures cause evaporation
- Disinfected levels deplete when evaporation combines with large numbers of people
- Bacteria grow making RWIs
- Primary causes of bacterial outbreaks
  - Uncontrolled water levels
  - Insufficient levels of disinfectants
  - Lack of cleaning leading to the formation of biofilms

Aerosols and Droplets

- Spread from some common artificial water systems
- Faucets, building air-conditioning units, hot tubs not drained after use, water fountains, water tanks and heaters, etc.
- Prevention: avoiding stagnation (stops biofilms forming)

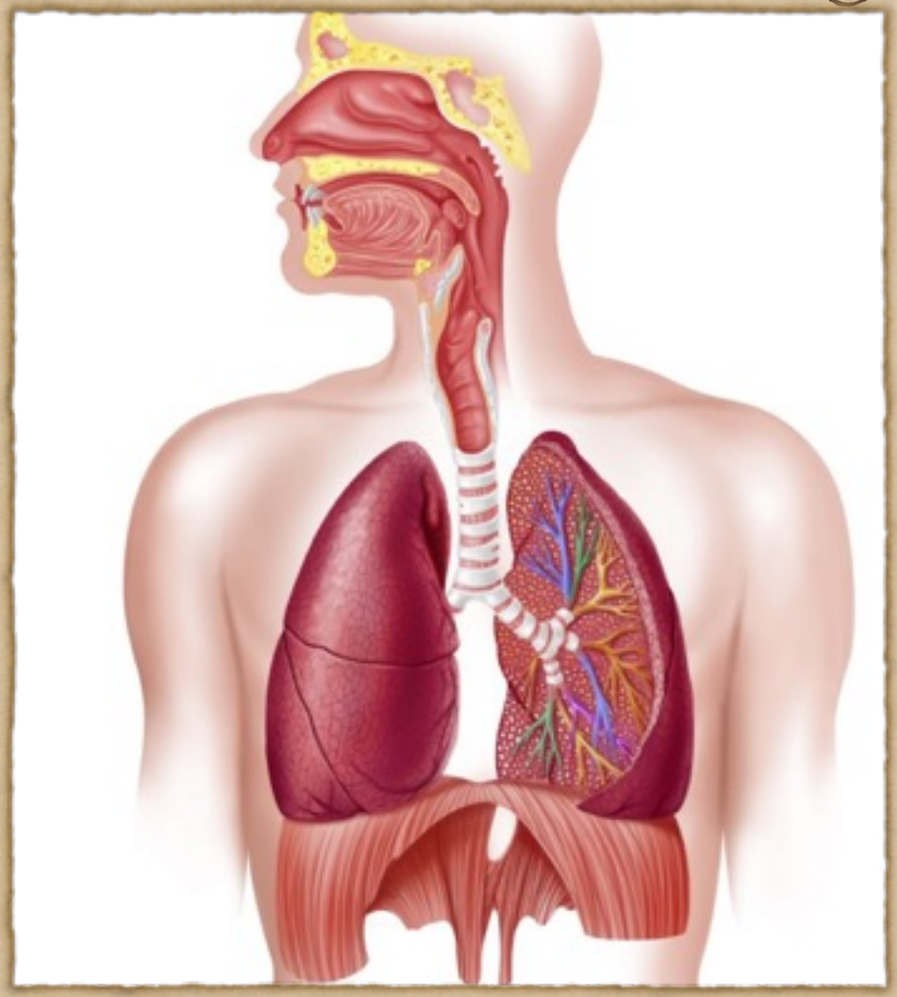


How Legionella deals with different environmental settings

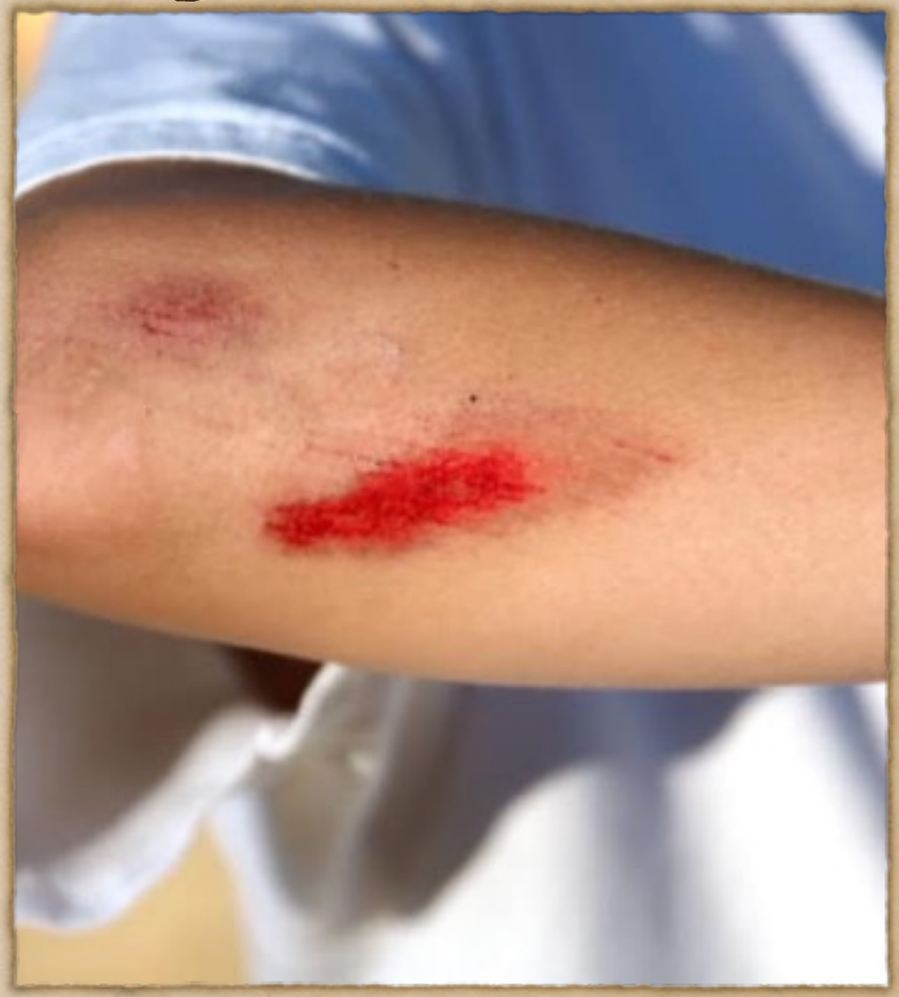
- Entering a temporary noncultivable state until favourable conditions come
    - Cell division decreased, metabolic activity is maintained
- Form biofilms for protection outside hosts and create nutrient gradients
- Type II secretion system: effectors produced obtain nutrients/survive
- Genes for survival and replication: lpg0730 and lpg0122 - encodes parts for ATP binding cassette (ABC) transport complex (function of complex unknown)

#2 - Entry

Areas that Legionella can enter the body through



Respiratory tract through inhalation (common route)

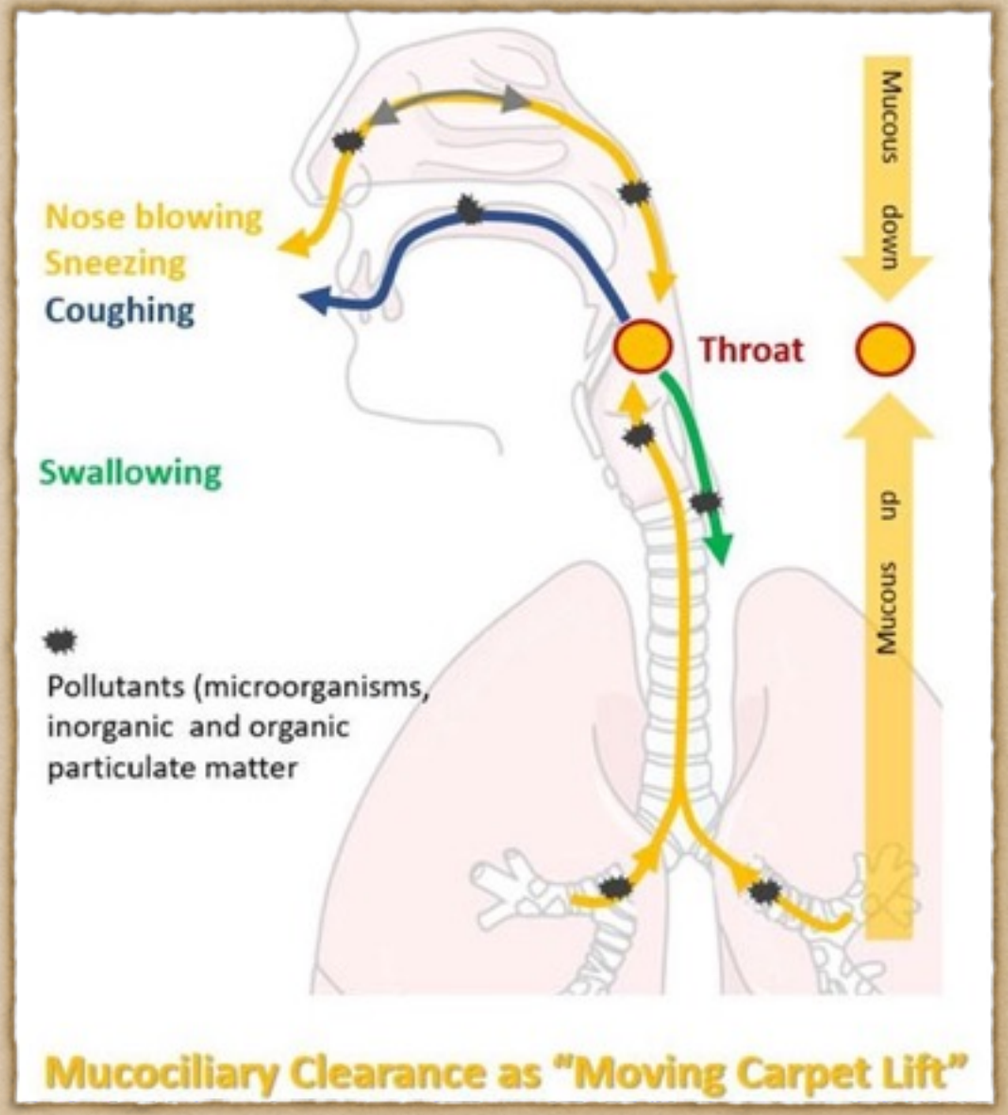


Superficial wounds (very rare)

Respiratory System: the more common entrance route

- Legionella usually cleared out by mucociliary action in upper respiratory tract, but patients with weak immune systems have impaired function in this area (ie. asthma patients like Tom)

- Travels down to lower respiratory tract for infection.

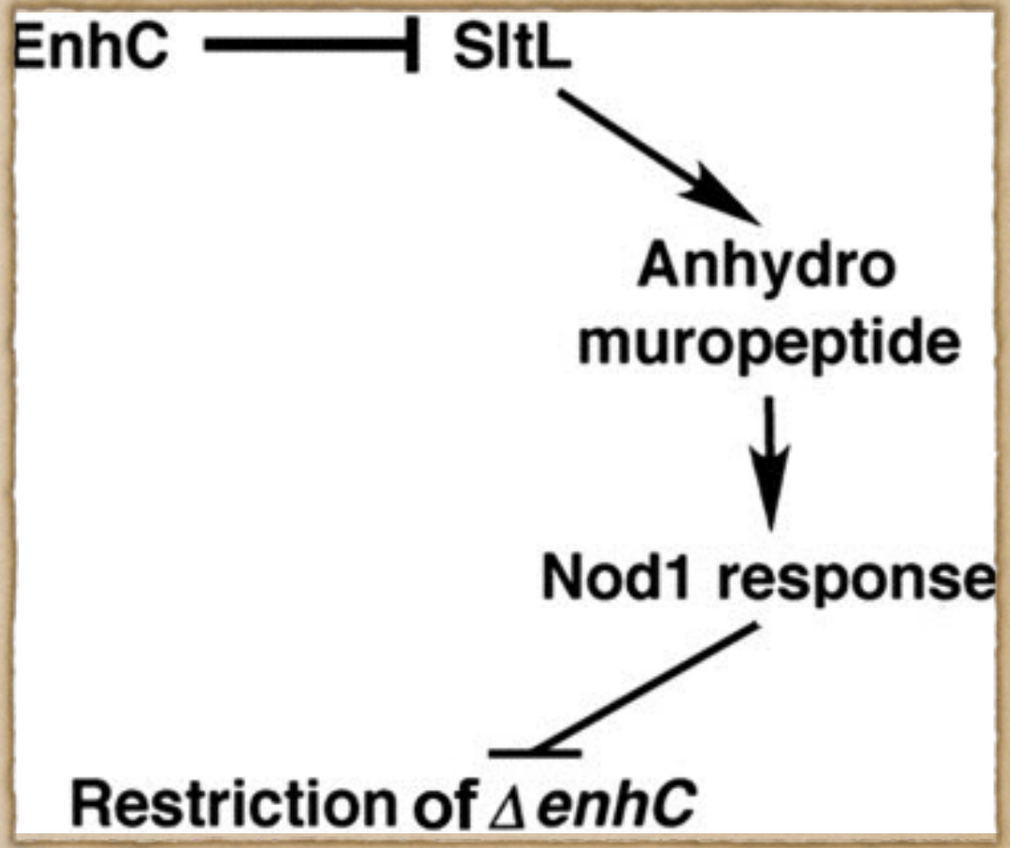


- Legionella are known to multiply intracellularly, but how?

- This is not well explained, but certain bacterial factors have been identified to play a role in its attachment and entry into the host cell.

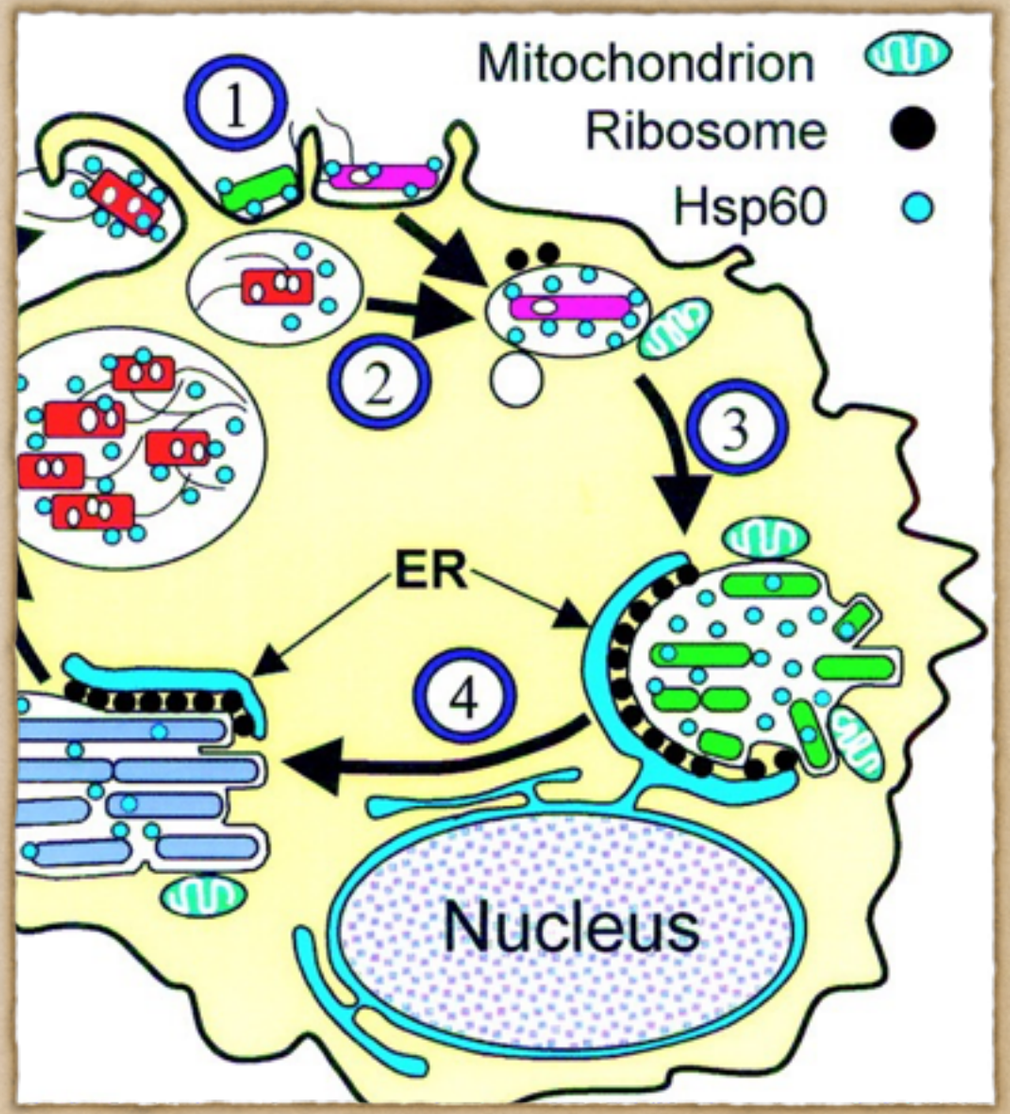
EnhC

- Periplasmic protein
- Role in efficient replication inside the macrophage
- Maintain cell wall integrity
- Reduces NOD1 on host cell, decreasing innate immune recognition of bacteria



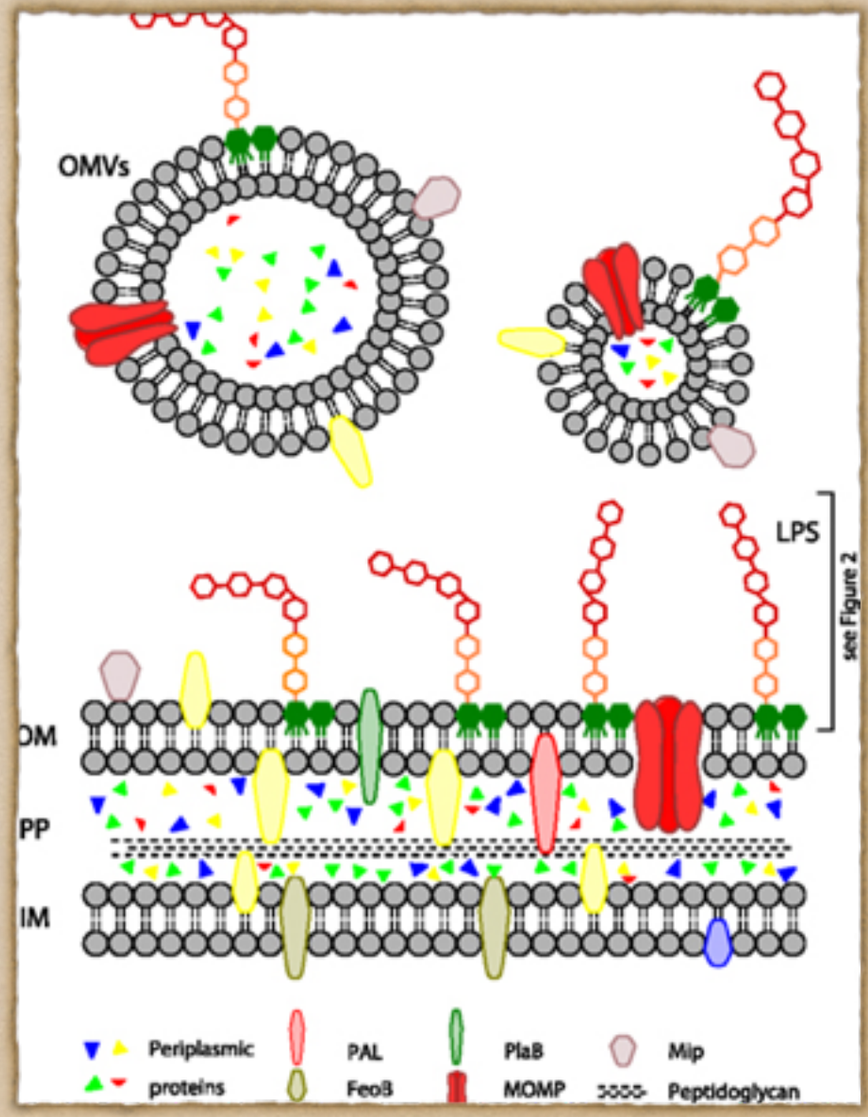
Hsp60

- Important for entry
- One of the most abundant proteins synthesized by the bacteria.
- Mediates phagocytosis by modulating the function of macrophages



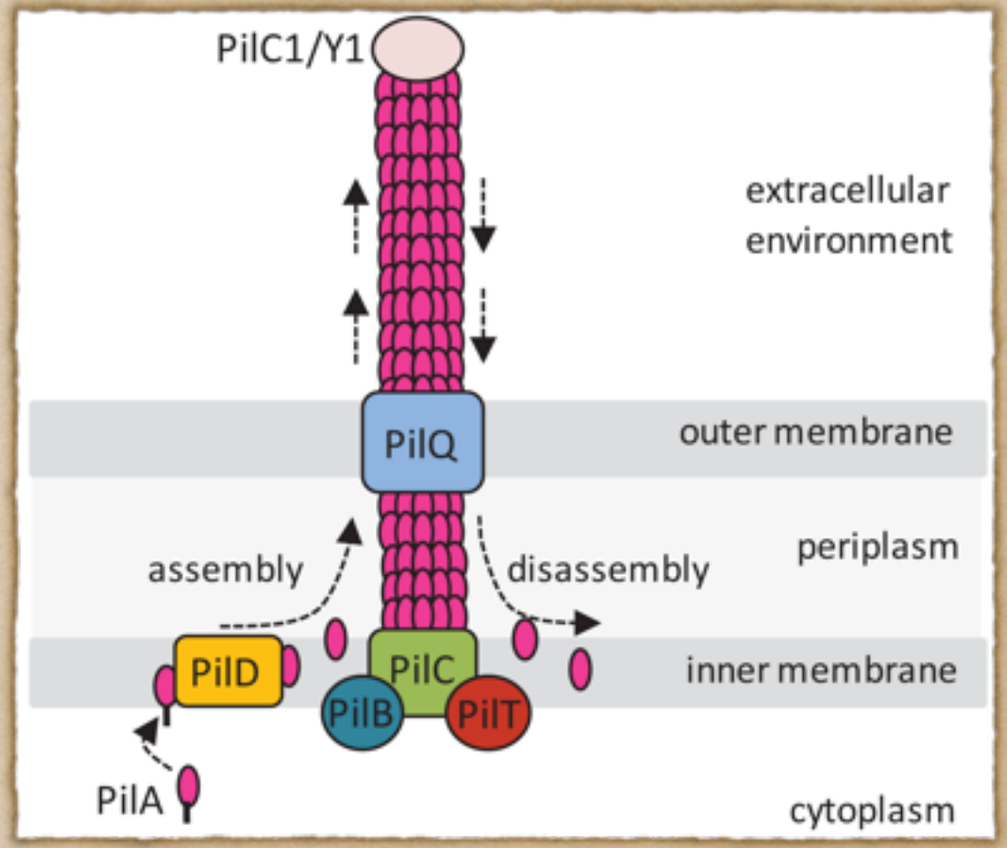
MOMP

- Attachment to host cells
- Mediates the fixing of C3 to the bacterial surface by the alternative pathway of the complement system
    - More convenient entry using CR1 & CR3 for phagocytosis



Type IV Pili

- Role in both attachment and entry.
- Adherence of bacteria to host tissue to facilitate invasion
- Also plays a role in biofilm formation
    - Promotes adherence and survival in unfavourable conditions

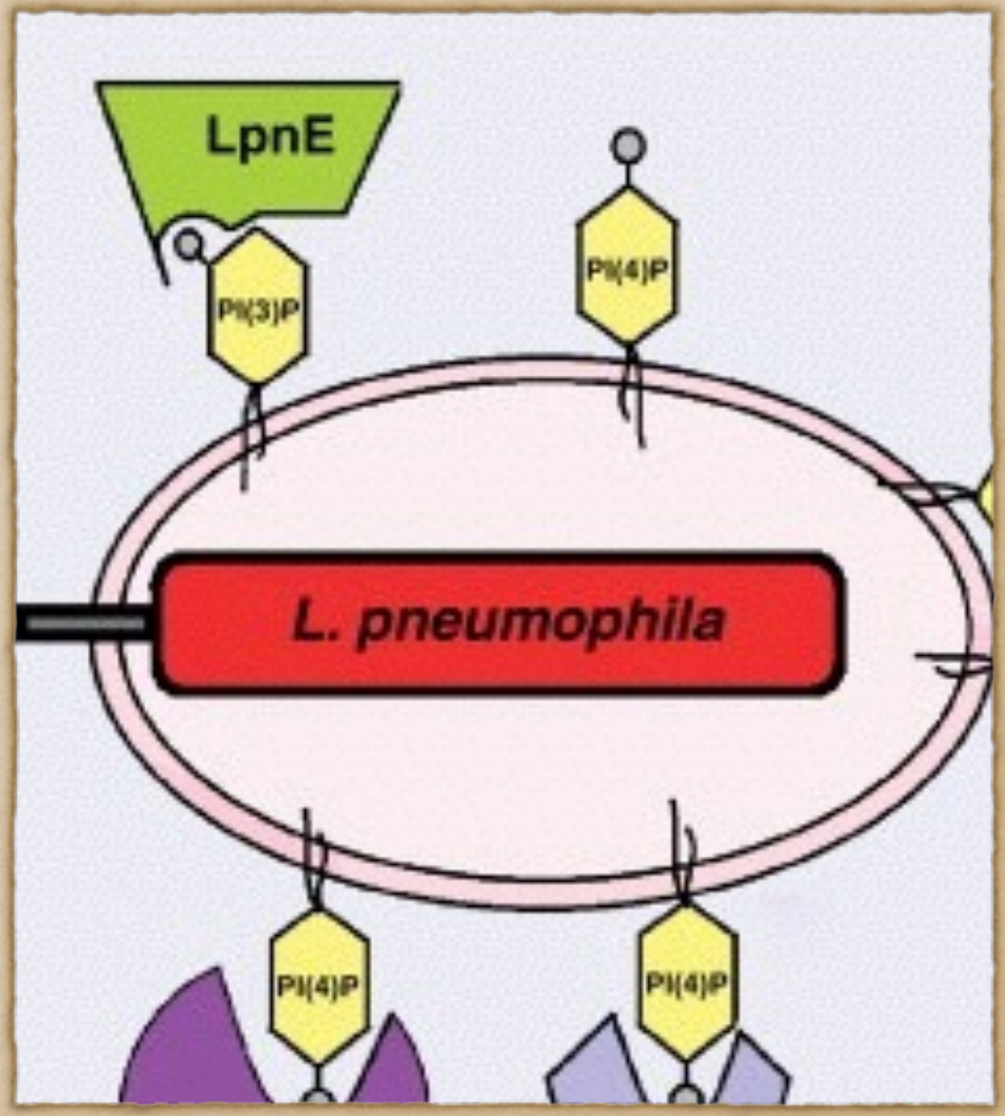


LpnE

- Plays a contributing role in mediating bacterial attachment

- Encoding gene: lpnE, required for full entry into macrophage and for efficient infection.

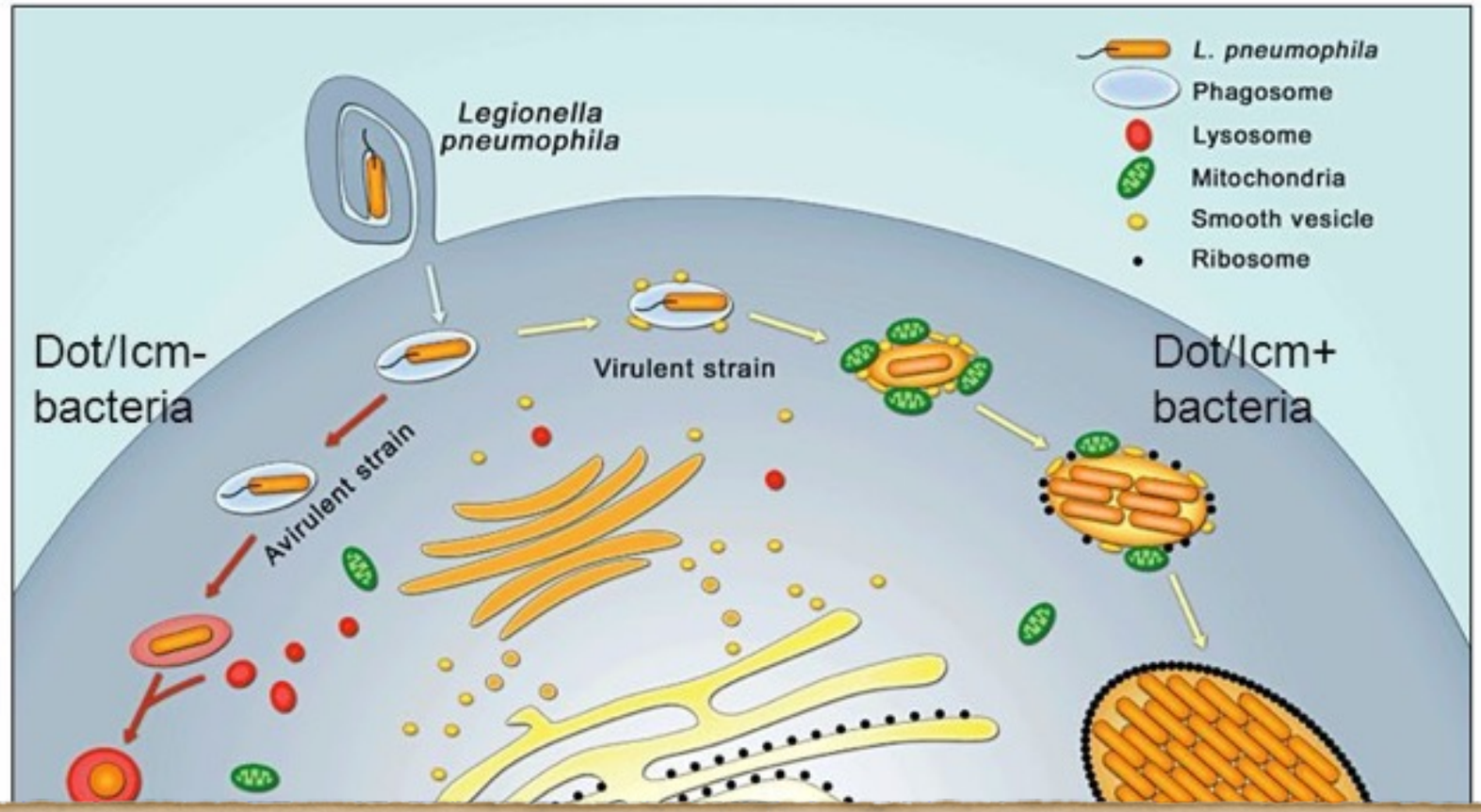
- Also has a role in trafficking the Legionella vacuole



Other Factors

- RtxA: role in bacterial attachment, but mechanism still not well understood.
- LcI and LadC: contribute to adherence and invasion of host cells

L. pneumophila (Pathogenesis)



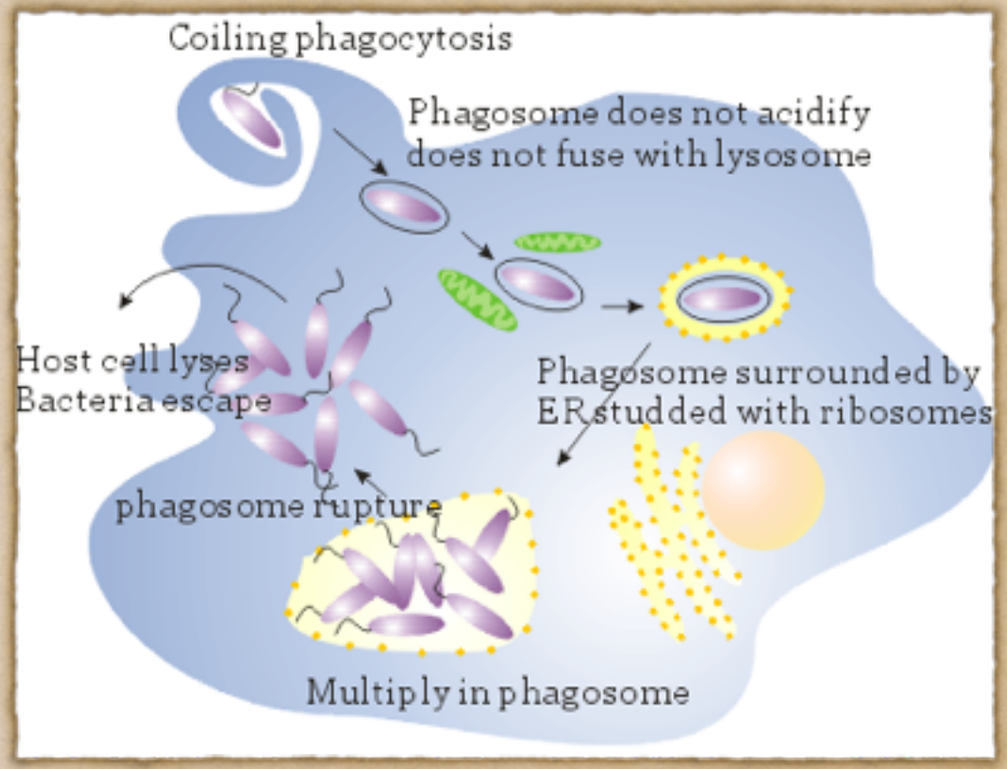
Summary of Attachment & Entry

Bind to alveolar macrophages of host through complement receptors, and enter by being engulfed into phagosomal vacuoles.

#3 - Multiplication & Spread

Engulfment

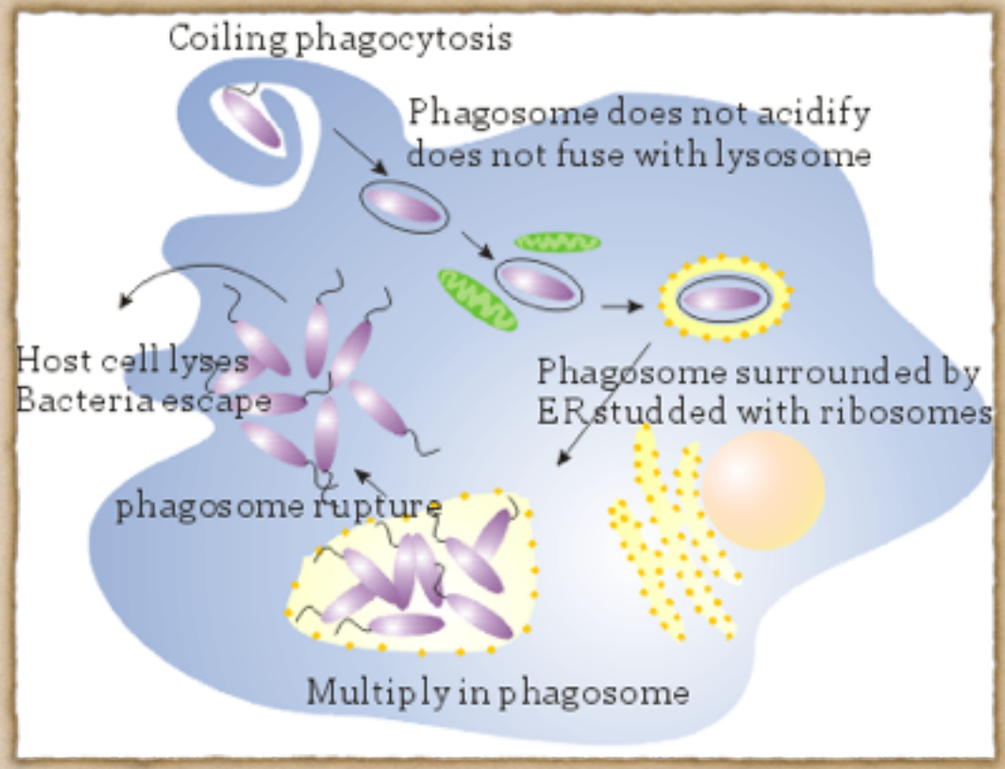
- Macrophage provides permissive environment for the bacteria.
- Bacteria phagocytoses, enters vacuole and alters composition
  - ie.modification of organelle trafficking to allow nutrient supply
- Organism inhibits the oxidative burst, reduces phagosome acidification



L. pneumophila taken up by macrophages via "coiling phagocytosis"

Engulfment

- Rearrangement of actin filaments to form the coiling asymmetrical pseudopod encircling the extracellular pathogen
- Effectors translocated into cytosol, powered by ATP hydrolysis and PMF
- Dot/icm effector VipA: polymerize microfilaments for bacteria engulfment
- Bacteria taken up and a nascent phagosome (not yet inherently bactericidal) is formed
- Cronin: assist with phagosome formation



Phagosome: site of replication for bacteria

Necessary Secretory Systems for Multiplication Process

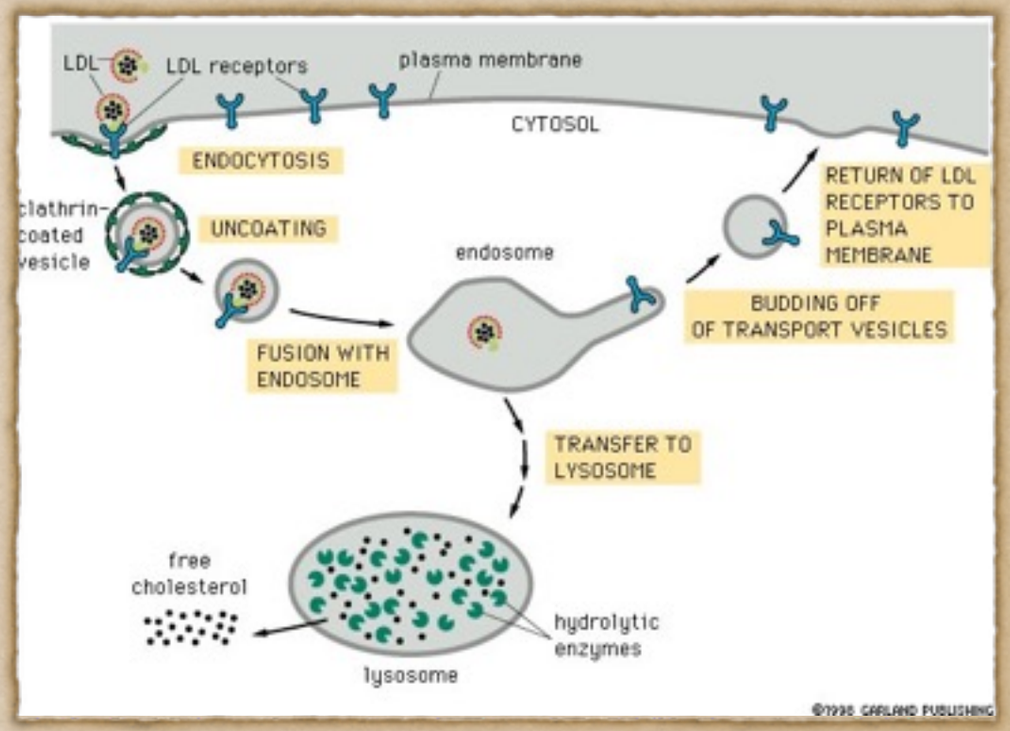
1. Type IV secretion system: necessary for the intracellular growth

   - 24 essential genes for the host infection - i.e.pilE (pilin protein), pilD (prepilin peptidase), which are both important for bacterial growth, mak (macrophage killing) mil (macrophage-specific infectivity loci) or pmi (protozoan macrophage infectivity)

2. Legionella vir homologs (Lvh): important, but not sufficient on its own (Type IV is needed alongside Lvh)

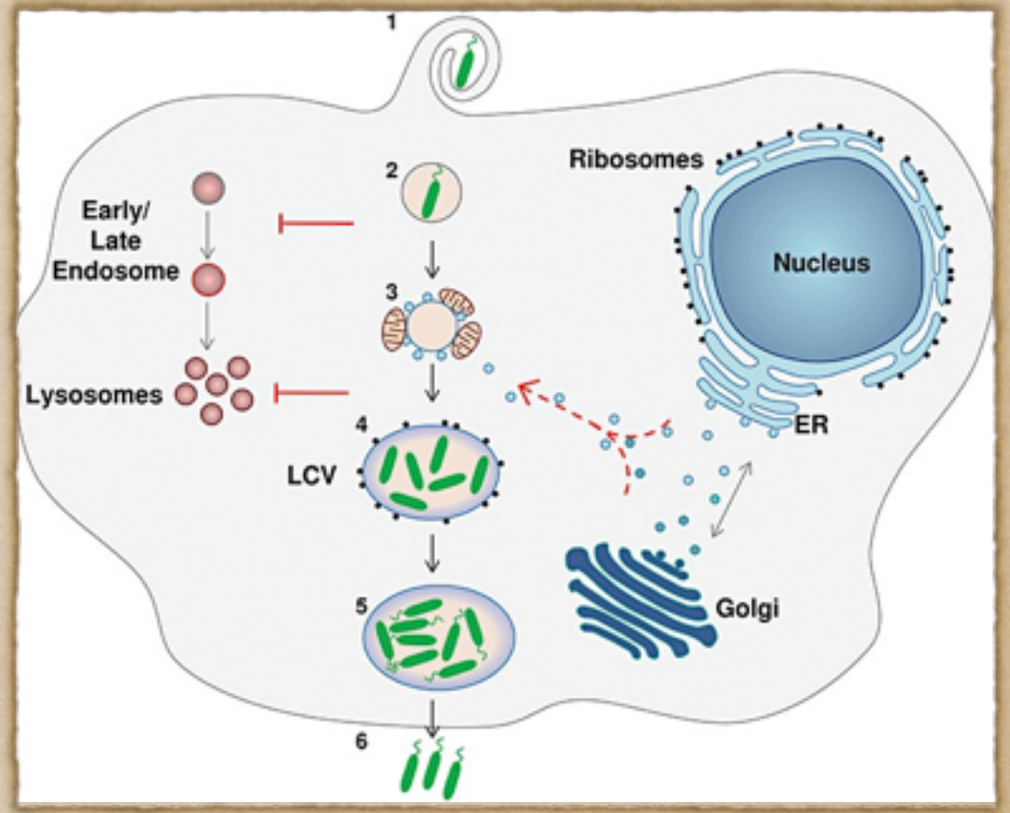
Avoiding endocytic pathway: What usually happens

- Phagosome fuse with early endosomes (acquire small GTPases Rab5)
- Matures and increases acidity (lowering pH), obtaining LAMPS (lysosome-associated membrane proteins) after interacting late endosome
- Fuses with lysosome to form phagolysosome, bactericidal killing and digestion occur
- Process disrupted when Legionella engulfed



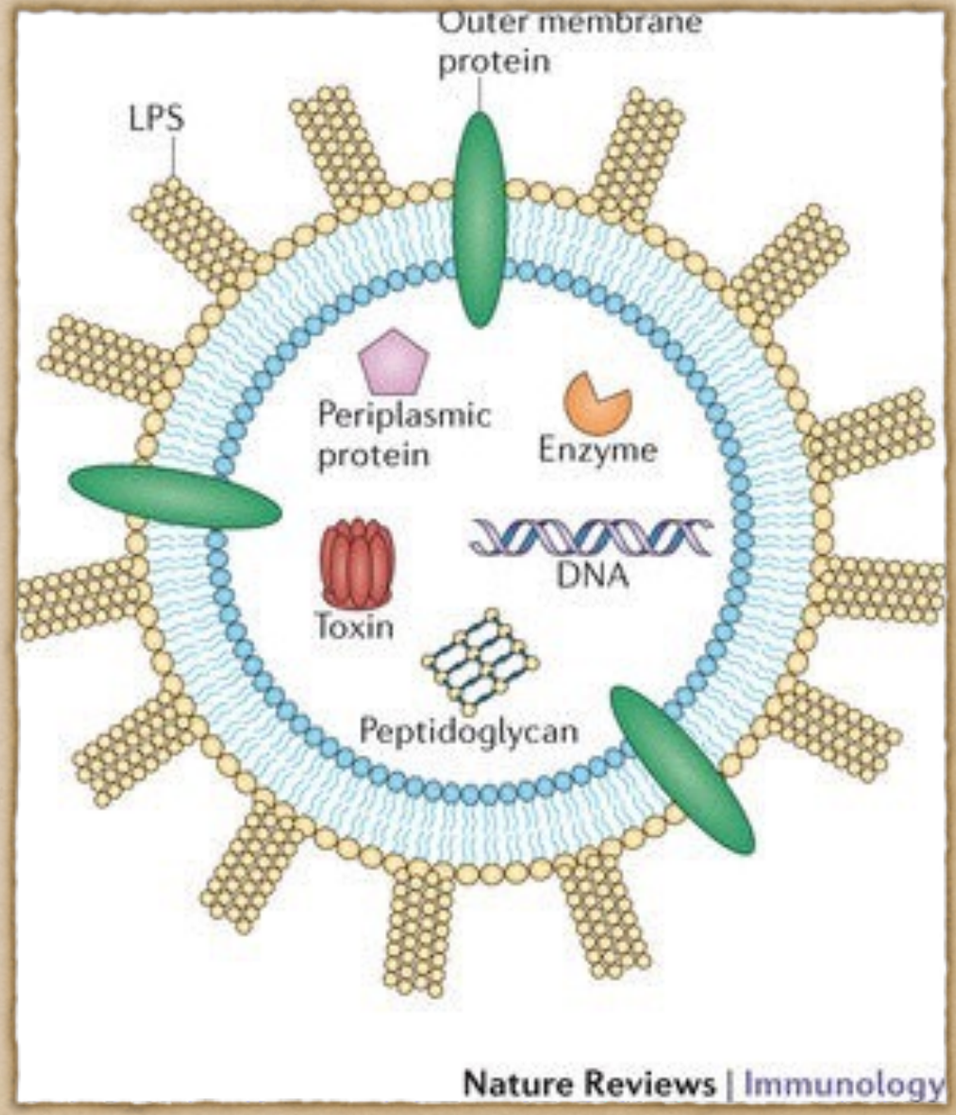
Avoiding endocytic pathway: What happens with Legionella

- Neutral pH is maintained in the phagosome for the first 6hrs after phagocytosis of bacteria
  - Regulating v-ATPase in the macrophage may be significant to avoiding early stage acidification
- Later stages (18hr): LCV acidified and take on lysosomal characteristics.
- Note: Bacteria replicating in macrophages are resistant to low pH environments



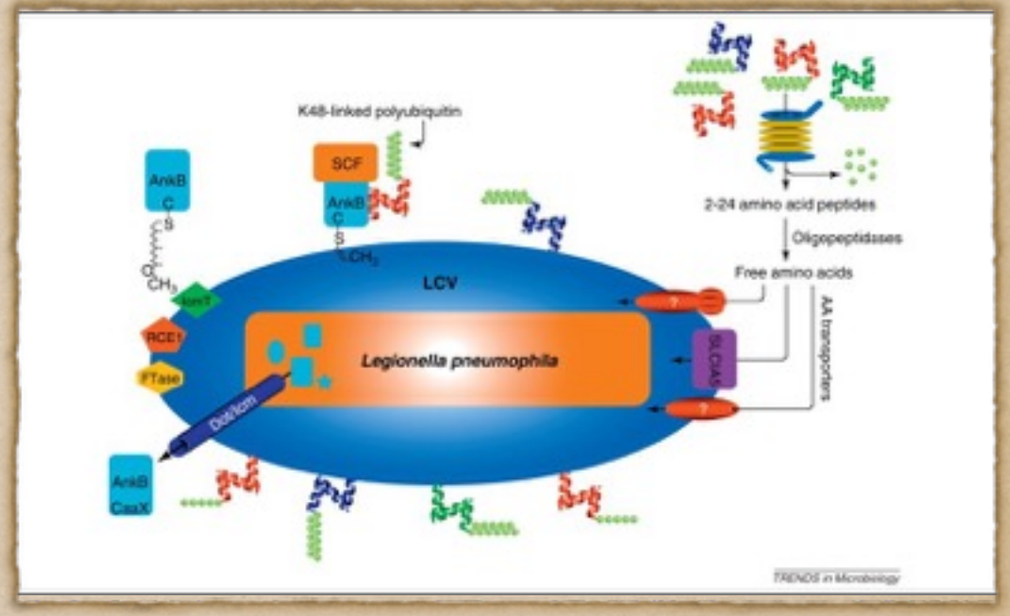
Avoiding endocytic pathway: Outer membrane vesicles (OMV)

- Shed from the outer membrane of gram negative bacteria
- Reported to block phagosome-lysosome fusion
- Can deliver packages of molecules (includes viral proteins)
- LPS from OMV may also contribute to arresting phagosome development



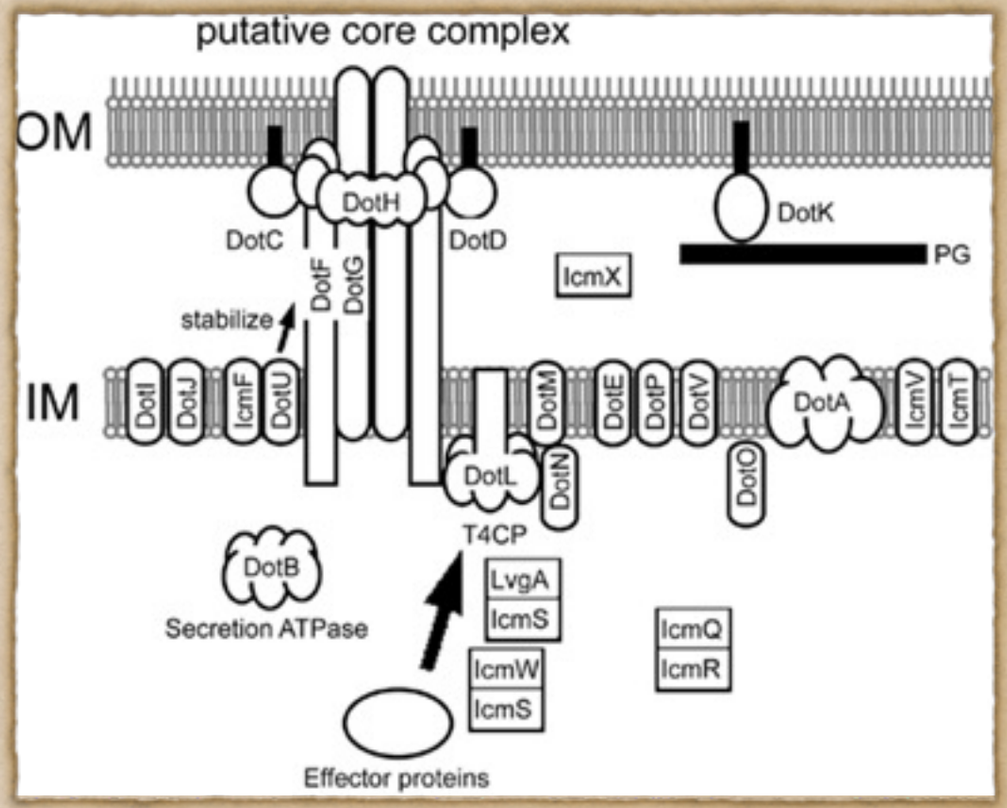
Formation of the LCV for Replication

- Legionella manipulates remodels the phagosome into a replication permissive LCV, avoiding fusion with lysosome
- LCV used for replication, analogous to rough ER used for replication
- Early infection stages: LCV surround by smooth vesicles and mitochondria
- Hijacks secretory vesicles exiting the ER and adopting luminal contents of vesicles that typically cycle between ER and Golgi
- Secretory vesicles leaving ER fuse with LCV



Formation of the LCV for Replication

- Dot/icm type IVB secretion system transports effector proteins to host cytoplasm across membranes
  - Important for forming the LCV but not necessary when replication compartment is established (will be useful, however, for inhibiting host cell apoptosis and egress from the macrophage)
  - Effectors participate in manipulating vesicle and membrane trafficking at this stage
  - Help with mitochondria recruitment to LCV (less known about this process)
- Targets host systems involved in vesicle and membrane transport to modify the vacuole into LCV



Formation of the LCV for Replication: Recruiting PIs

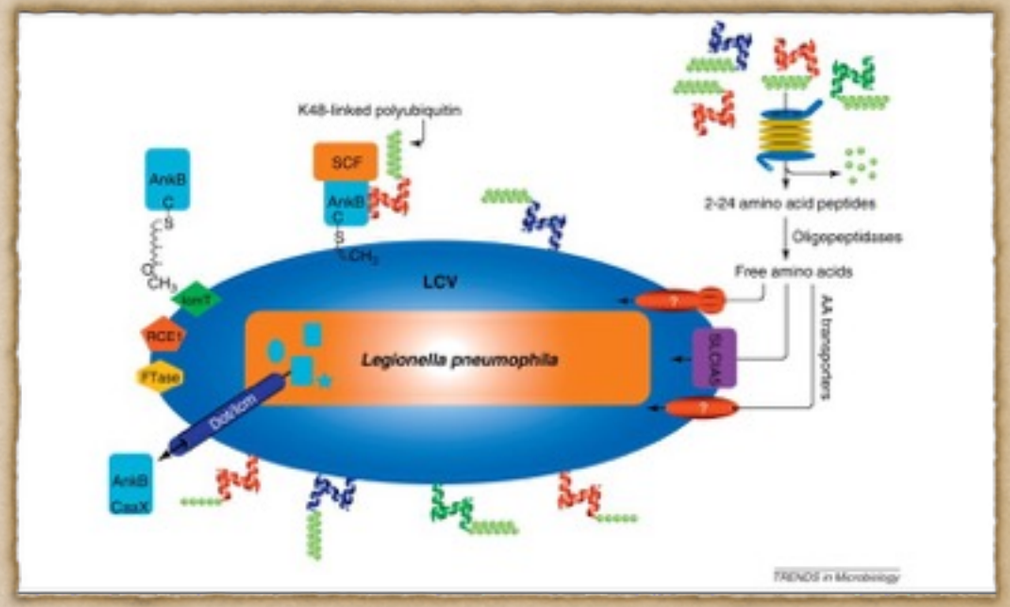
- Bacteria exploit phosphoinositide lipids (PI) involved in membrane transport when LCV is forming
- Sid family effectors enhance level of PI(4)P on LCV membrane, likely enhancing attachment of ER-derived vesicles to the former plasma-membrane of LCV
- PI also provides important attachment site for effectors on cytoplasmic face of LCV

Formation of LCV for Replication

- LCV takes on characteristics of ribosome studded rough ER, ER-derived vesicles and mitochondria surrounding LCV will decrease and get replaced by ribosomes on LCV membrane

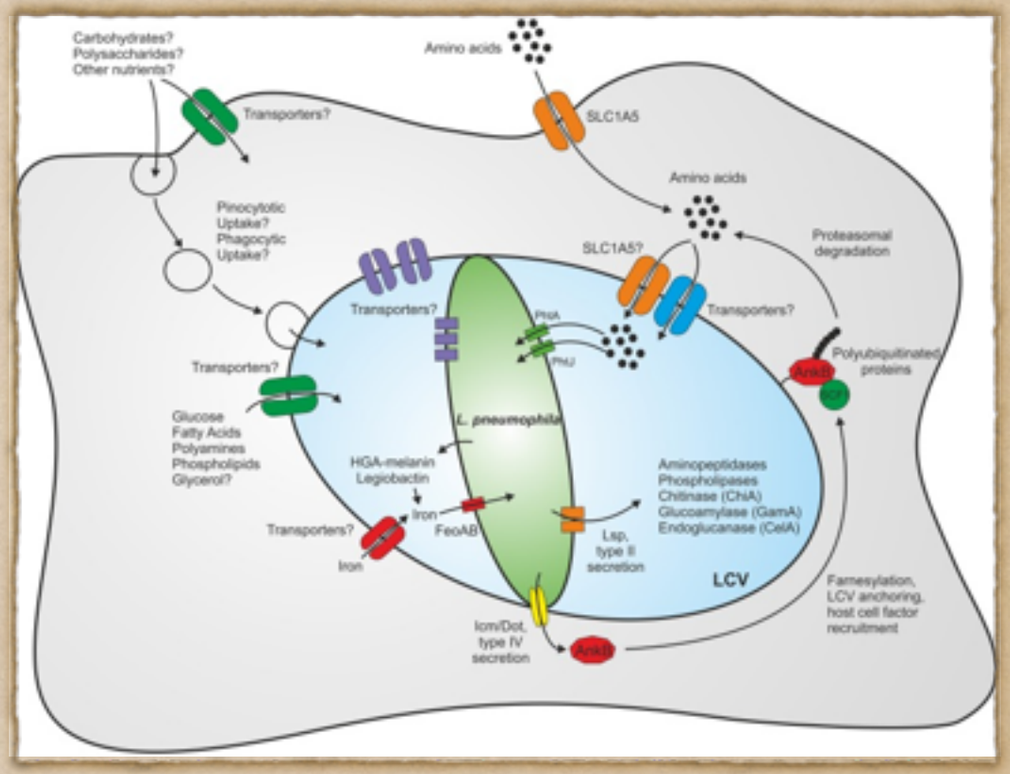
- Initial remodeling of the LCV has rendered it sufficiently ER-like to allow for spontaneous ribosome attraction

- Replication of L.pneumophila begins approx. 4-10 hours after phagocytosis and after establishment of the RER-like former phagosome



Formation of LCV for Replication: The nutrient environment

- L. pneumophila recruit ubiquitinated proteins to LCV mediated by AnkB
- Degradation of the ubiquitinated proteins may provide a source of amino acids for the replicating bacteria
- Synthesize amino acid transporters "phagosomal transporters", necessary for intracellular replication.
- Iron acquisition also important for intracellular replication
- Autophagosomes may recruit to the LCV as a source of nutrients for replicating bacteria



Formation of LCV for Replication: Does it really escape the lysosome suppressing pathway?

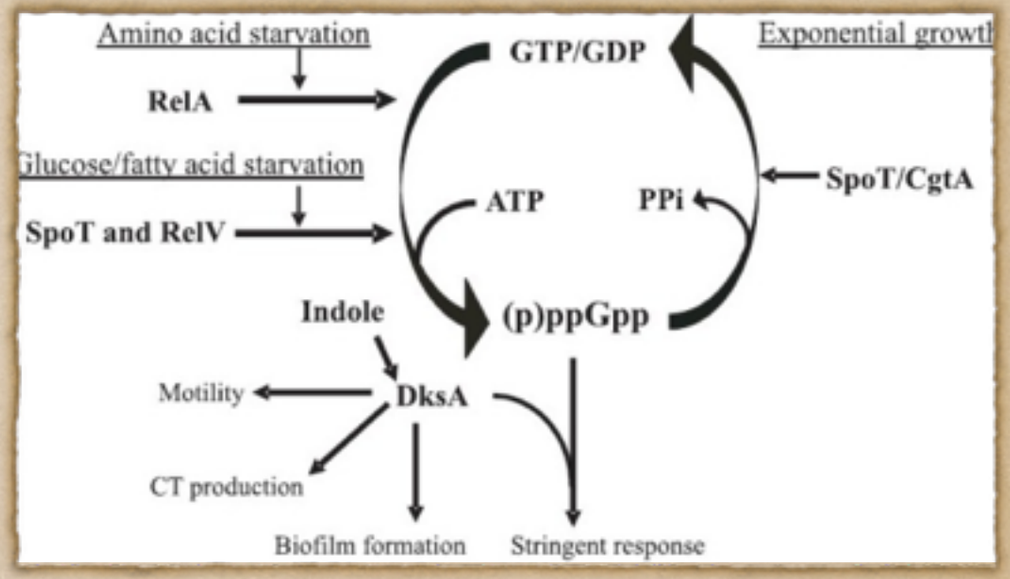
- Vacuoles that bacteria replicate in seem to adopt a phagolysosome character
- Late bacteria are more acid-resistant than it previously was, and the acquisition of lysosomal proteins appears necessary for survival and late replication
- Recruitment of the usual proteins in the endocytic pathway still takes place, except later than usually
- Possible idea: vacuole will fuse with a lysosomal vacuole, where availability in nutriment is high, and replication occurs there until amino acid supply has been fully consumed

Formation of LCV for Replication: Time for spreading

- Amino acid starvation induces accumulation of ppGpp triggering the start of the transmission process: cytotoxicity, osmotic resistance, motility, and final evasion from the lysosomal pathway

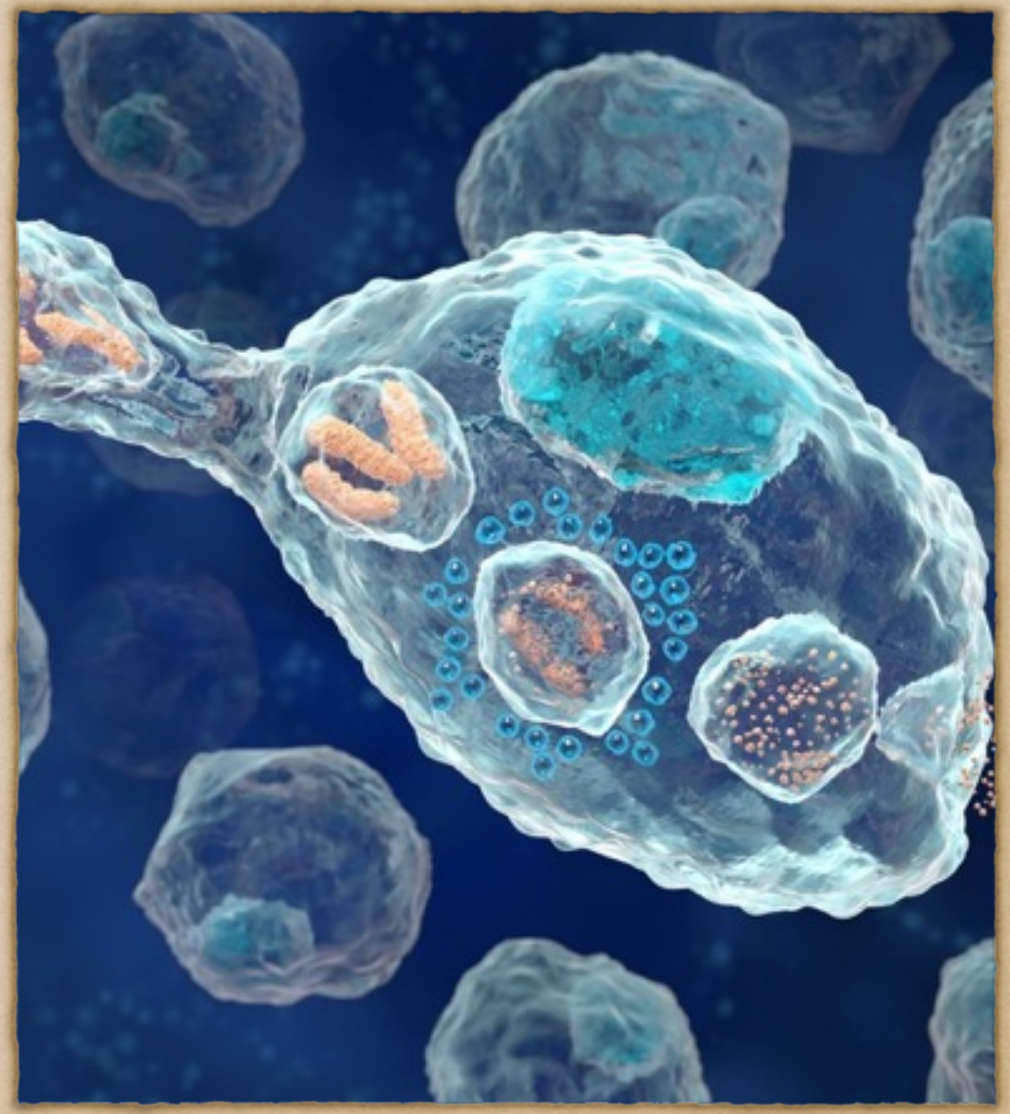
- Regulators of gene expression influence transformation from replicative into motile infectious phase bacteria

- Cytotoxins production is induced and lead to host cell lysis



Secondary Infection

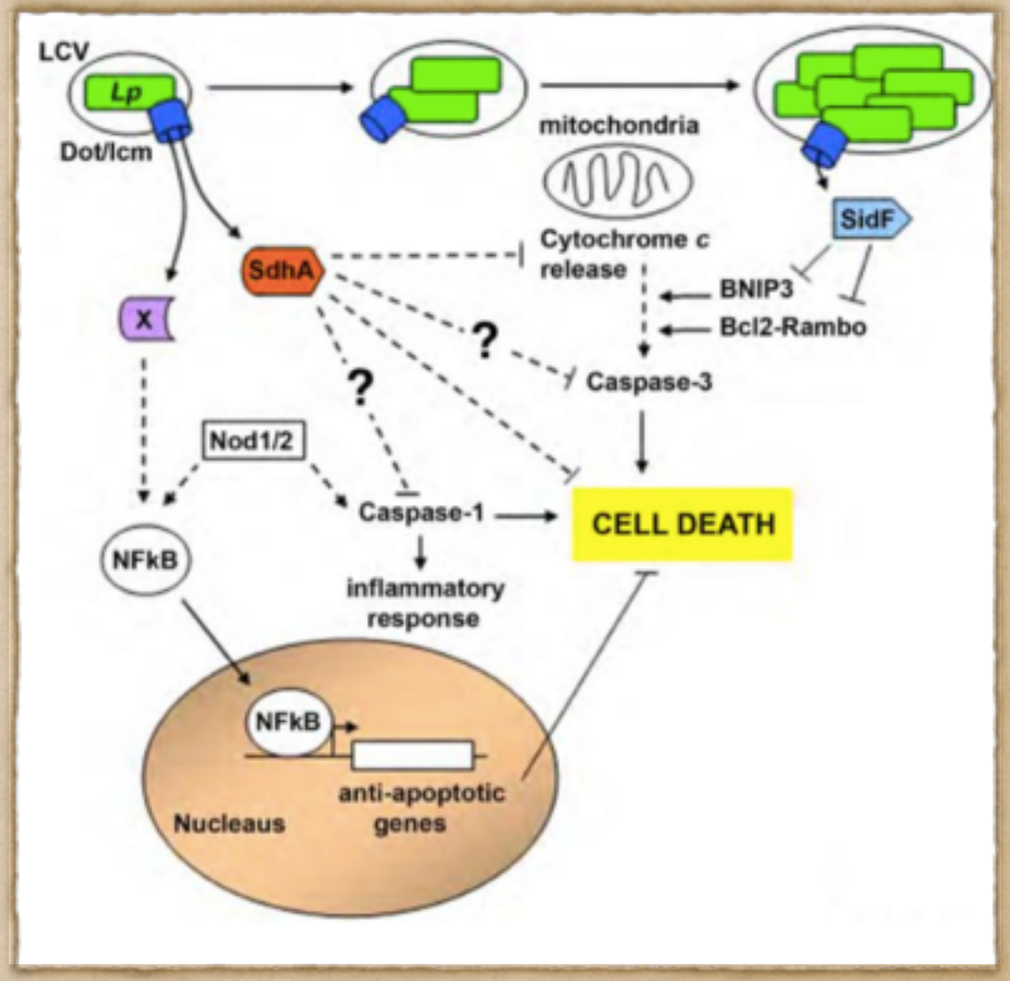
- Any other manifestations that occur outside of the lungs are considered secondary infections.
    - ie. Splenomegaly (enlargement of spleen) and spleen rupture, pericarditis, wounds, joint infection, and CNS.
- Infect extrapulmonary sites via hematogenous spread (distribute by bloodstream) after pulmonary focus
- May travel to other sites using the macrophages that they are replicating in, getting deposited to other potential infection sites after macrophage lyses



#4 - Bacterial Damage

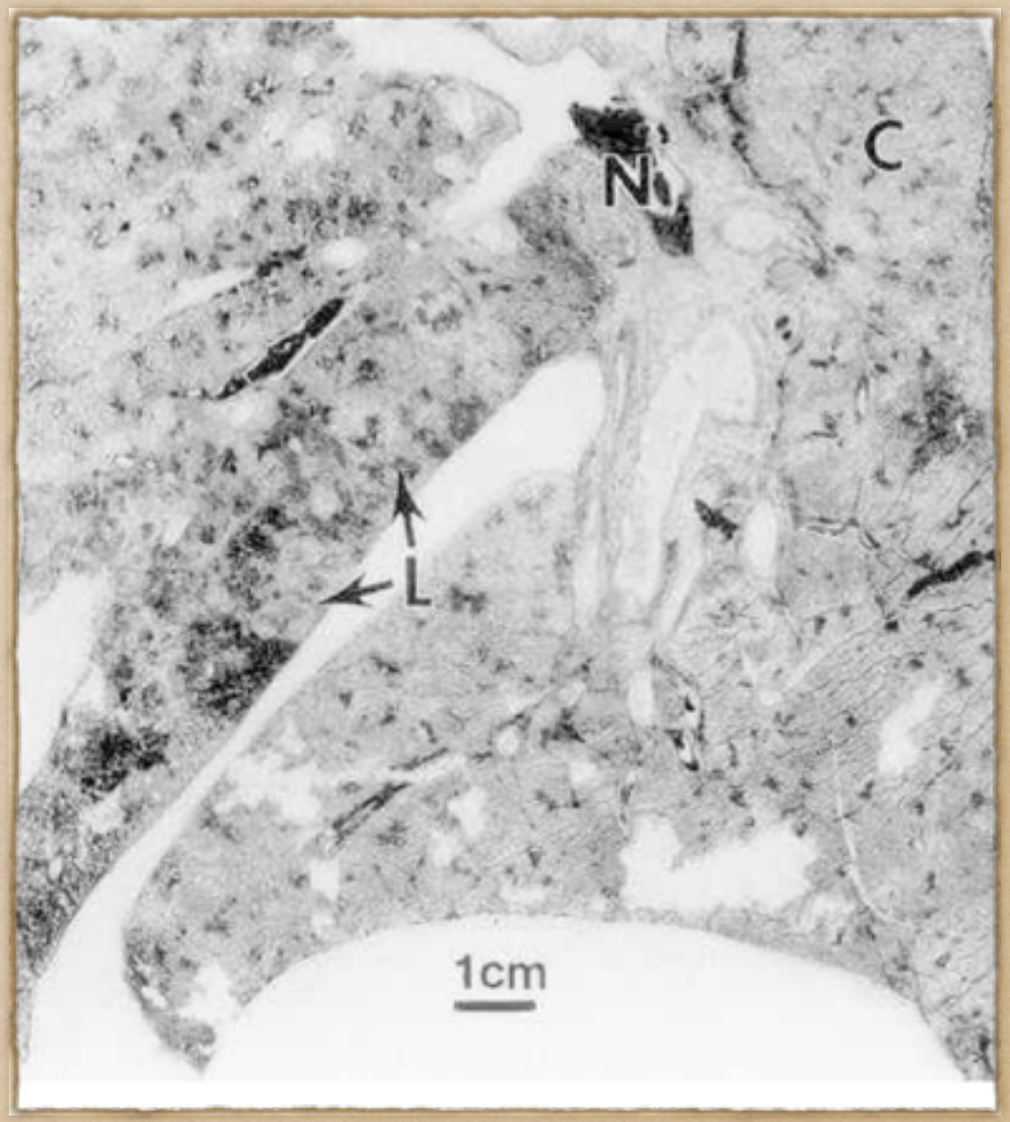
Direct damage: host cell death from exhaustion of resources

- Host cell killing is non-apoptotic and can occur at low doses of bacteria
- Uncontrolled cell lysis can increase damage and spread to neighbouring cells
- Bacterial factors may also cause tissue damage
- Microbial components associated with cell death not yet identified
  - Possible one: flagellin, helping to promote caspase1-dependent cell death.
- Primary site of host cell damage: destruction of pulmonary tissues and cells



Indirect Damage: Host Defence Responses

- Toxic oxygen dependent killing directed by neutrophils - leads to accumulation of protein-rich fluid flooding alveolar space
- Inflammatory response involving recruitment of neutrophils and macrophages
    - Cell factors (ie.IL-1) released from monocytes: fever
    - Influx of innate immune cells: increase in body temperature & other symptoms
- Leaky capillaries allow for influx of serum, increases deposition of fibrin in alveoli
- Result: destruction of air spaces, compromise respiratory functions



- Untreated Legionnaires' disease can lead to more serious complications
- ie. Respiratory failure, septic shock, multi-organ dysfunction