Legionella Pneumophila: Bacterial Pathogenesis

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ENCOUNTER

Where is *L. pneumophila* found?

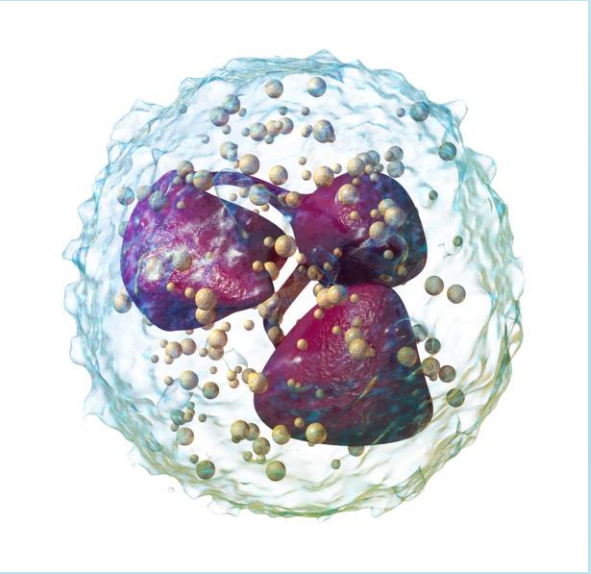
- Globally distributed
  - *Canada, Japan, US, Europe, Australia, New Zealand and Singapore*

Where is *L. pneumophila* found geographically?

- Naturally in freshwater environments such as rivers and lakes
- Other water systems such as hot tubs, distribution systems and pools
- Can grow at temperatures between 20C and 50C (ideal for hot tubs)
- Moist, soil

Where is *L. pneumophila* found hostwise?

- grow in amoebae and ciliated protozoa
  including Hartmanella, Acanthamoeba and Naeglaria species
- inside **alveolar macrophages** and **neutrophils** in human hosts



What are the bacterial characteristics that leave it suited to these places of residence?

1. **Type II secretion system**: aids in survival and obtaining nutrients during stressful situations
2. **Genes for survival and replication**: lpg0730 and lpg0122 - encodes parts for ATP binding cassette (ABC) transport complex (function of complex unknown)
3. **Entering a noncultivable state**: under unfavourable conditions, L. pneumophila enters a state where no cell division takes place
4. **Biofilms:** provide protection for when outside a host and allow bacteria to create a nutrient gradient

How did our patient come into contact with *L. pneumophila*?

Tom likely came into contact with L. pneumophila while using the hot tubs on the cruise.

Entry

What facilitates the entry of the bacteria into the human host?

- *L. pneumophila* usually enters the host through the inhalation of contaminated aerosols water
- It is also possible for the bacteria to enter the host by infecting a wound, though this is very rare
- *L. pneumophila* is usually cleared by the mucociliary action of the upper respiratory tract
- Any bacteria not cleared by the mucociliary action will travel down to the lower respiratory tract and begin an infection
- An immunocompromised host, such as a person with diabetes or lung diseases, is at a higher risk of infection from *L. pneumophila*

Entry from Bacterial Perspective

It is unknown exactly how the bacteria are internalized by eukaryotic cells, however research has found a number of bacterial factors that play a role in attachment and entry:

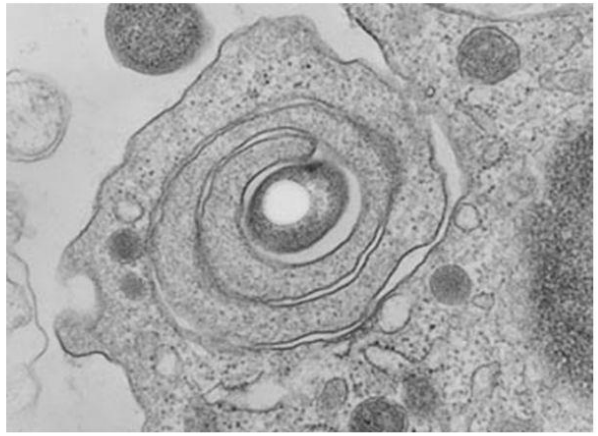
Bacterial Factor	Function
EnhC	Facilitates evasion from recognition by NOD1 of host cell; maintains cell wall; aids replications inside macrophage
Hsp60	A chaperone protein which modulates the function of macrophages to allow for phagocytosis(entry) of bacteria
MOMP (major outer membrane protein)	Facilitates fixation of C3 (bacterial complement component) to CR1 and CR3 (host complement receptors); this allows for attachment and phagocytosis of bacteria
Type IV pilus	Aids in adherence of bacteria to host tissue; promotes formation of biofilms
LpnE	Aids in attachment to host cell; the LpnE gene is required for complete entry and efficient infection
RtxA	Aids in bacterial attachment and entry
Lcl and LadC	Proteins which contribute to adherence to and invasion of host cell

Multiplication and Spread

Engulfment

- *L. pneumophila* is an **intracellular pathogen** during its replication phase
- Alveolar macrophages take up L. pneumophila through **coiling phagocytosis – a process in which the bacteria induce rearrangement of the actin filaments to create** asymmetrical pseudopods
- dot/icm effector, **VipA**, is involved in actin polymerization which aids the engulfment of *L. pneumophila*
- actin binding proteins called cronins are sometimes also recruited

Coiling phagocytosis: human monocyte ingesting *Legionella pneumophila*



Avoiding the Endocytic Pathway

- **Neutral pH** is maintained for 6 hours to **avoid early acidification**

  Achieved by **r**egulating **v-ATPase** in the macrophage

- **Shedding of outer membrane vesicles (OMVs)** from *L. pneumophila* has been reported to block phagosome-lysosome fusion

  Lipopolysaccharides found on OMVs also help arrest phagosome development

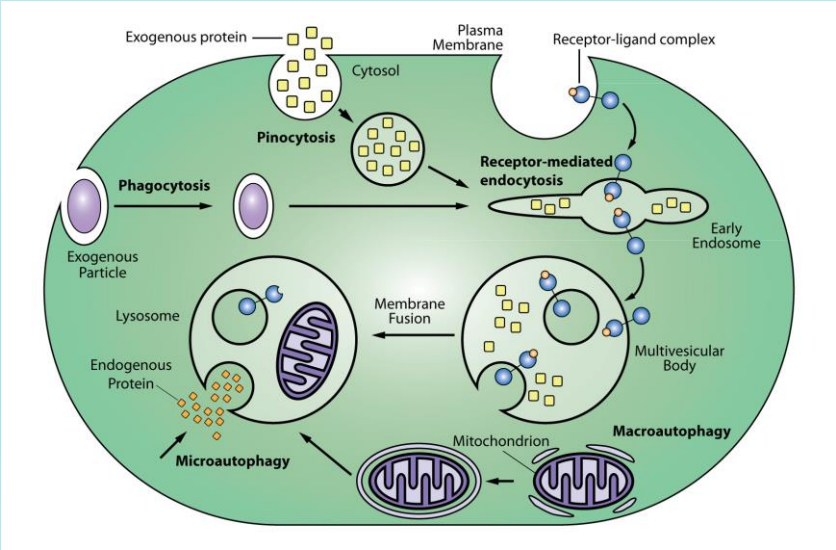
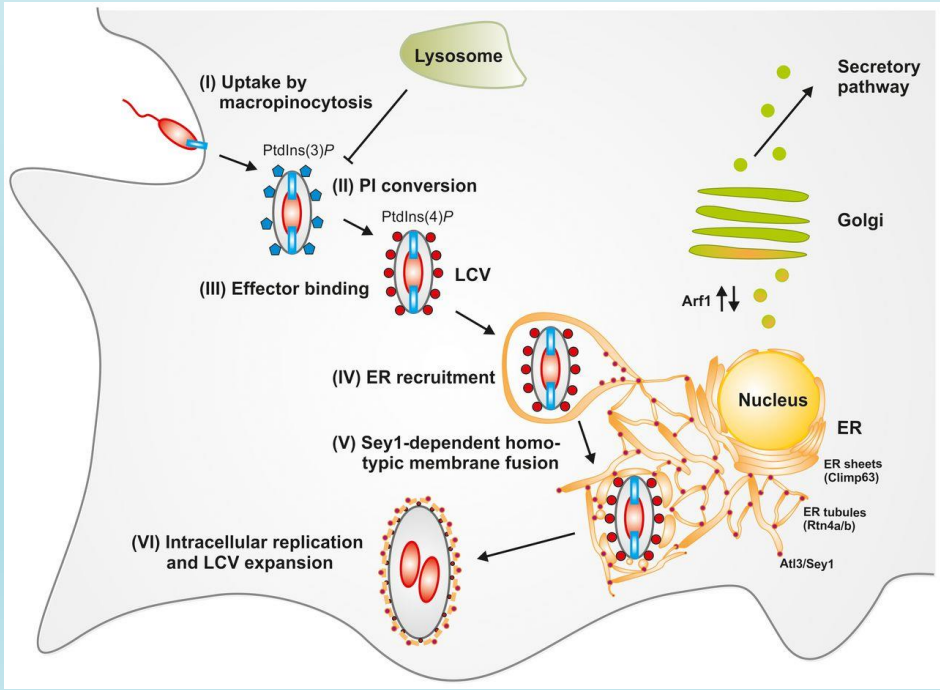


Diagram of normal endocytic pathway

Formation of the LCV for Replication

In order to replicate, *L. pneumophila* must remodel the phagosome into a replication permissive **LCV**

- **dot/icm type IVB secretion system** has effectors which modify the vacuole to take on characteristics of the **ER**
- **SidM/DrrA and RalF** mediate recruitment of the host proteins **Rab1 and Arf1** facilitate the tethering and fusion of ER-derived vesicles with the LCV
- **SNARE Sec22b complex** is also recruited to **enhance fusion** with ER vesicles
- **Phosphoinositide lipids (PIs)** is likely involved in **attachment of ER-derived vesicles** and is an attachment site for effectors such as **SidC**
- **Dot/Icm secretory system** is necessary for ribosome recruitment



Multiplication – Inside the Phagosome

- **Type IV secretion system** gene contains 24 essential genes for the host infection

  Virulence factors include pilE, pilD, mak (macrophage killing), mil (macrophage-specific infectivity loci) or pmi (protozoan macrophage infectivity)

- Requires **adequate nutrient levels**

  An effector, **AnkB** is used to recruit ubiquitinated proteins, which are later degraded by host proteasomes; this provides *L. pneumophila* with a source of amino acids

  Nutrients are obtained by manipulating **host autophagy system (energy of recycled macromolecules)**

- Use **siderophores and transport proteins** to obtain iron necessary for replication
- Secrete molecules that can **upregulate iron uptake in low-iron conditions**
- Dot-Icm system works to inhibit apoptosis while bacteria are replicating

Secondary Infection

- secondary infections are rare but have been observed in other organs such as the **kidneys and lymph nodes**
- *L. pneumophila* are able to spread through out the body by entering the **bloodstream** (hematogenous spread)
- it is hypothesized that they use the phagosome as vehicle for transportation in the body, and once the phagosome lyses, the bacteria infect nearby organs

Bacterial Damage

Direct Damage – due to *L. pneumophila* actions

- **Uncontrolled lysis** causes bacteria to spread and effect neighbouring cells and organs
- **Bacterial proteases** can cause tissue damage
- Destruction of **pulmonary tissues and cells**, including alveolar macrophages.
- **Flagellin** could be promote caspase1-dependent cell death.

Indirect Damage – due to host immune response

- Damage to lung epithelial and endothelial cells by neutrophils as a result of toxic O_2 concentrations
- Pneumonia due to accumulation of fluids in alveolar space

  Results in difficulty breathing
- Inflammatory response due to the detection

of *L. Pneumophila*

- Accumulation of protein rich fluid