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Legionnaire’s Disease

- *L. pneumophila* was first found as cause of pneumonia in 1976 at Legionnaires convention in Philadelphia (221 infected, 34 died)
- This disease has highlighted the need to keep air conditioners clean: incidence increased dramatically with central air conditioning in large buildings
- Invades and replicates within a protective phagosome inside alveolar (LCV: Legionella-Containing Vacuole)

Legionella pneumophila is a bacterial pathogen that infects alveolar macrophages and replicates inside a specialized organelle that is morphologically similar to the endolysosomal system. While the host cell's endosomes are known to contain proteases that kill many pathogens, *L. pneumophila* has evolved a strategy to avoid degradation and to multiply within a protective phagosome (LCV). This phagosome is decorated by several hours and replicates within the phagosome. Replication results in the formation of a specialized organelle that can support bacterial replication.

**Are Rabs involved in Legionella-Containing Vacuole?**

- Rab1 is a GTPase involved in fusion of ER-derived vesicles with preGolgi and the Golgi apparatus
- Rab1 recruits factors necessary for the tethering and fusion of ER-derived vesicles with target membranes
- This fusion process requires the pairing of v-SNARE and t-SNARE
Rab1 is important for Legionella’s intracellular growth

Rab1 promotes transport and fusion of ER-derived vesicles containing Sec22b
ER-derived vesicles deliver sec22b to the LCV

*Immunoelectron microscopy assay*

- sec22b on vesicles attached to the LCV
- sec22b on the vacuole membrane

**Wild type Legionella**

No sec22b on dotA mutant Legionella

Sec22b, transmembrane protein 🔄 ER-derived vesicles fuse with the LCV

The ratio of sec22b:membrin affected the delivery of Sec22b to the LCV

<table>
<thead>
<tr>
<th>Sec22b: Membrane Ratio (Ratio of Plasmid Transfected)</th>
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<tbody>
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- Sec22b is important for establishment of the Legionella replicative organelle!

**Overproduction of SNAREs had different effects**

<table>
<thead>
<tr>
<th>SNARE</th>
<th>Effect on Legionella Intracellular Growth</th>
<th>SEAP Secretion of Alkaline Phosphatase</th>
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<tbody>
<tr>
<td>Sec22b</td>
<td>did not affect</td>
<td>did not affect</td>
</tr>
<tr>
<td>Membrin</td>
<td>interfered</td>
<td>interfered</td>
</tr>
<tr>
<td>vAMP4</td>
<td>Enhance</td>
<td>interfered</td>
</tr>
<tr>
<td>ARF1(T31N)</td>
<td>interfered</td>
<td>interfered</td>
</tr>
<tr>
<td>Membrin-Sec22b</td>
<td>Enhance</td>
<td>interfered</td>
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<td>Membrin-VAMP4</td>
<td>interfered</td>
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</tr>
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**Summary**

- Rab1 is specifically recruited by LCV, which is dependent upon Dot/lcm system
- Rab1 mutant Interferes with intracellular replication of legionella
- Rab1 function is associated with the delivery of the sec22b to the LCV
- Immunoelectron microscopy assay indicated that sec22b is delivered by ER-derived vesicles
- Host ER–Golgi Rab1 and SNARE proteins play an important role in formation of the Legionella replicative organelle.
One of the functions of certain antibody molecules known as IgG is to stick antigens such as bacterial proteins and polysaccharides to phagocytes. The “tips” of the antibody, the Fab portion, have a shape that fits epitopes, portions of an antigen with a complementary shape. The “stalk” of the antibody is called the Fc portion and is able to bind to Fc receptors on phagocytes. Also, when body defense pathways known as the complement pathways are activated, one of the beneficial defense proteins made is called C3b. C3b binds by one end to bacterial surface proteins and by the other end to C3b receptors on phagocytes. The IgG and C3b are also known as opsonins and the process of enhanced attachment is also called opsonization.

Note: The drug Brefeldin A inhibits activation of the ARF protein by inhibiting nucleotide exchange, and thereby inhibits budding of COPI vesicles.

despite in vitro studies demonstrating functional identity between Rab1a and Rab1b, there may be an undetected difference that could change the experimental interpretation.

colony forming unit (CFU)

SEAP: secretory alkaline phosphatase;
Fc receptor for IgG (FcγRII).

Traffic: ER to Golgi to Lysosome

2. Types of Coats:
   b. CopI: Made of coatamer subunits. Mediates retrieval of proteins from Golgi to ER (retrograde transport). COPI vesicles transport ER resident proteins with KXXX or RRXX signals. Uses GTP binding protein ARF (as does clathrin).
   Note: The drug Brefeldin A inhibits activation of the ARF protein by inhibiting nucleotide exchange, and thereby inhibits budding of COPI vesicles.
   c. CopII: Mediates forward movement of vesicles from ER to Golgi (anterograde transport). Regulated by a GTP binding protein Sar1. Budding of COPII is not inhibited by Brefeldin A (which is specific for ARF).