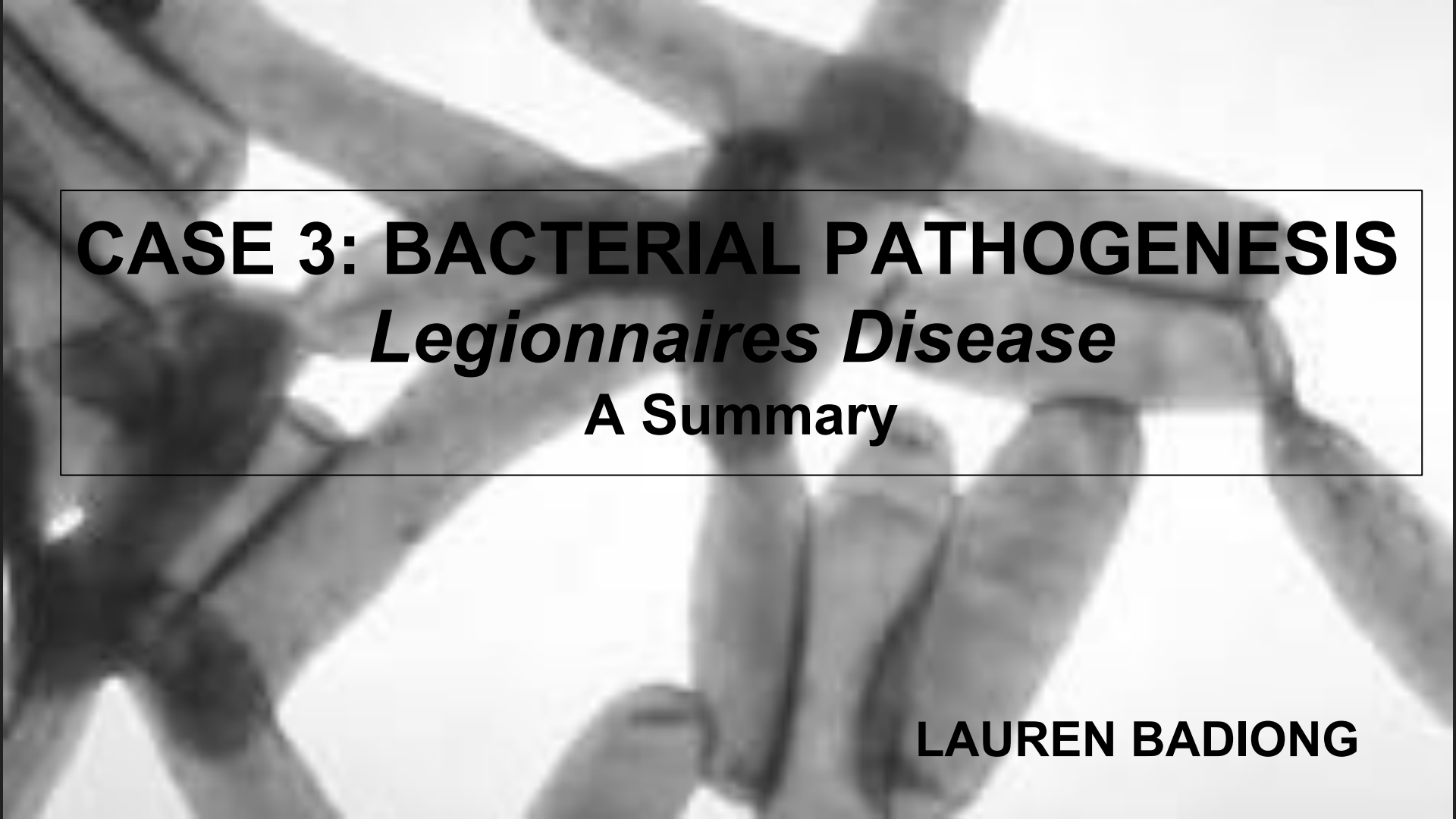


CASE 3: BACTERIAL PATHOGENESIS

Legionnaires Disease

A Summary

LAUREN BADIONG

ABOUT THE ORGANISM: RESIDENCE

- Fresh water environments: rivers and lakes
- In amoeba and ciliated protozoa
  - Hartmanella, Acanthamoeba, and Naeglaria species
  - Would reside here for protection and growth during infection
- Geographically:
  - USA, Canada, New Zealand, AUstralia, Japan, Singapore, and in Europe
  - Some studies state high numbers of cases in France, Italy, SPain, and increasing incidence in the USA

- Environmental Conditions
  - Optimal growing temperature for *L. pneumophila* is 35C, but tolerates temperatures from 20C to 50C → So they like hot tubs and pools

ABOUT THE ORGANISM: RESIDENCE

- What is it about Hot tubs?
  - Bacterial outbreaks in hot tubs can result from uncontrolled water temperatures, insufficient levels of disinfectants, and lack of cleaning → encouraging biofilm formation
  - Significant source of aerosols, facilitating entry into the host

- Bacterial Characteristics for Survival
  - Temporary Non-Cultivable state: cell division is decreased, but metabolic activity is maintained until favourable conditions return (coping with stressful conditions with differing temperatures, pH, nutrient availability, etc.)
  - Ability to form Biofilms: provide protection when outside a host and allows creation of nutrient gradients
  - Type II Secretion System: produces effectors that allow *L. pneumophila* to obtain nutrients and survive successful conditions

BACTERIAL ENTRY INTO THE HUMAN HOST

- Begins with the inhalation of contaminated aerosols
- Entry through superficial wounds can happen, but is very rare
- Immunocompromised Individuals: makes individuals more susceptible because they have less of an ability to blear the organisms through mucociliary clearance

- Process of internalization of bacteria by eukaryotic cells is not clear
- Bacterial factors identified to play a role in attachment and entry include:
    - EnhC, Lcl, Hsp60, MOMP, type IV pili, LpnE, RtxA, and LadC

BACTERIAL ENTRY INTO THE HUMAN HOST

EnhC: periplasmic protein that helps with efficient bacterial replication in macrophages

Hsp60: abundant protein during growth that mediates phagocytosis by modulating the function of macrophages → enhancing bacterial entry

MOMP: helps in the attachment to host cells → mediates the fixing of C3 to the bacterial surface via the alternative pathway of the human host

BACTERIAL ENTRY INTO THE HUMAN HOST

Type IV Pili: involved in the attachment and the entry of the bacteria into the host cells

- ❏ Involves adherence of the bacteria to host tissues, allowing bacterial invasion
- ❏ Involved in formation and development of biofilms, promoting the adherence of the pathogen and its survival in various environmental conditions

LpnE: its encoding gene, lnpE, it required for full bacterial entry into macrophages, which can then lead to effective and successful infection of the human host

RtxA: unclear mechanisms, but known to play a role in bacterial attachment and entry

- ❏ Mutants without RtxA observed to have decreased attachment and adherence of bacteria to human cells

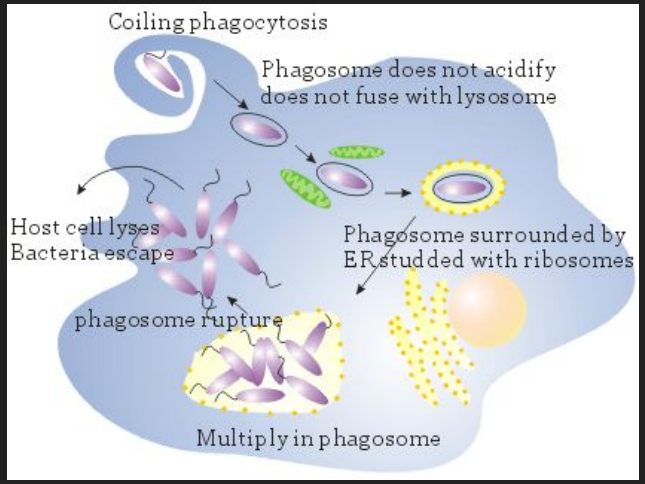
Lcl and LadC: proteins that contribute to adherence and invasion of host cells

***Ultimately, once complement receptors are bound to, bacteria can bind to host alveolar macrophages and can be engulfed into a phagosomal vacuole

BACTERIAL MULTIPLICATION AND SPREAD

ENGULFMENT

- *L. pneumophila* is phagocytosed, where it inhibits the host's oxidative burst and reduces phagosome acidification
- Bacteria multiplies intracellularly in human macrophages, including lung alveolar macrophages
- Bacteria uses the phagosome as a site of replication



LEGIONELLA LIFE CYCLE

- Occurs within the macrophages that engulf them
- Has a permissive environment for bacterial growth and replication
- Modification occur: organelle trafficking (to allow for bacterial nutrient supply) → bacterial becomes better-equipped for intracellular replication
- Modification may be linked to interactions with the mitochondria and the Endoplasmic Reticulum

BACTERIAL MULTIPLICATION AND SPREAD

PHAGOCYTOSIS

- Macrophages take up the bacteria via ‘COILING PHAGOCYTOSIS’
  - Legionella intake depends on its strain-host specificity
  - Bacteria induces the rearrangement of actin filaments, forming the coiling asymmetrical pseudopod encircling the extracellular pathogen
  - Effectors become translocated into the cytosol, powered by ATP hydrolysis and Proton-Motive Force (PMF)
  - Effectors act like an actin nucleator, that is highly involved in actin polymerization → microfilaments are polymerized for engulfment of the bacteria = phagocytosis
- CRONINS: and actin-binding protein is recruited to help with phagosome formation
- Multiplication Process, the Type IV secretion system:
  - Virulence Factors: pilE (pilin protein) and pilD (prepilin peptidase) are both involved in bacterial growth, macrophage killing, macrophage-specificity infectivity loci, or protozoan macrophage infectivity
  - Secretory System, Lvh (Legionella vir homologs): important for intracellular growth (but still needs the type IV secretory system for efficient growth)

BACTERIAL MULTIPLICATION AND SPREAD

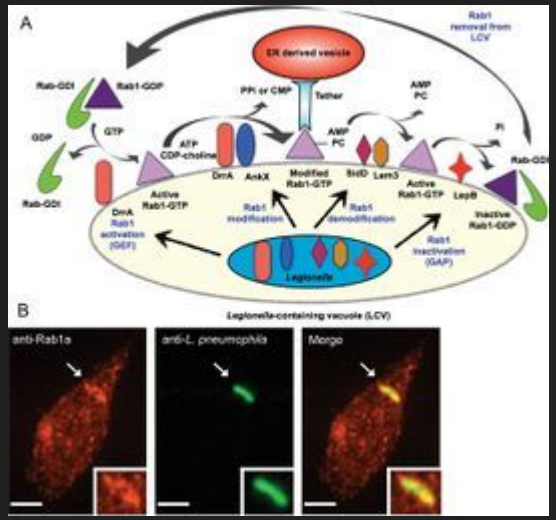
AVOIDING ENDOCYTIC PATHWAY

- Virulence system is encoded by 26 dot/icm genes (dot: defective in organelle trafficking; icm: intracellular multiplication)
  - For host cell entry, intracellular multiplication, and anti-apoptotic host cell signaling → Can also disrupt phagosome and host cell membranes
  - Phagosome fuses with early endosomes after phagocytosis (and acquires Rab5, small GTPases), and it continues to mature, becoming increasingly acidic → After, they obtain lysosome-associated membrane proteins (LAMPs) after interacting with late endosomes
  - Phagosome + late-endosome-like phenotype will fuse with a lysosome to form a PHAGOLYSOSOME (here, bactericidal killing and digestion occur) → Maturation will occur once the environment gets more acidic in the phagosome (done by proton transporters called vacuolar H+-ATPase (v-ATPase) → This process is disrupted when Legionella are engulfed
  - How it gets stopped: a neutral pH is maintained for the first six hours, and bacteria avoids early acidification increasing the chances for infection → The outer membrane vesicles (OMVs) also prevent phagosome-lysosome fusion

BACTERIAL MULTIPLICATION AND SPREAD

FORMATION OF THE LCV FOR REPLICATION

- Legionella mechanism: manipulation of the phagosome occurs, turning it into a replication permissive LCV (Legionella-Containing Vacuole), avoiding fusion with lysosome
- In early infection stages
  - LCV surround smooth vesicles and mitochondria
  - Hijacking of secretory vesicles exiting the ER occurs: adopt luminal contents of vesicles that typically cycle between ER and Golgi
  - After, the secretory vesicles leaving ER fuse with LCV



TOP DIAGRAM

- Shows the Rab1 activation pathway
- Occurs in infected macrophages

BOTTOM DIAGRAM

- Shows Rab1 presence at the bacteria location

- Transported effector proteins into host cytoplasm across membranes is accomplished by dot/icm type IVB secretion system
  - This is crucial for the formation of LCV, but not required once the replication compartment has been established
  - This also helps in inhibiting host cell apoptosis and egress from the macrophage
  - Effectors participate in manipulating vesicle and membrane trafficking at this stage
  - Facilitate with mitochondria recruitments to LCV (process not that known yet)
- Bacteria manipulates host systems involved in vesicle and membrane transport to modify the vacuole into LCV

BACTERIAL MULTIPLICATION AND SPREAD

FORMATION OF THE LCV FOR REPLICATION: RECRUITING PIs

- Bacteria takes advantage of Phosphoinositide Lipids (PI), which is involved in membrane transport when LCV is forming
- Sid Family Effectors upregulate the level of PI(4)P on LCV membrane
    - This enhances the attachment of ER-derived vesicles to the former plasma-membrane of LCV
- PI also becomes an important attachment site for effectors on cytoplasmic face of LCV

BACTERIAL MULTIPLICATION AND SPREAD

MORE ON LCV FORMATION FOR REPLICATION

- LCV takes the characteristics of ribosome studded rough ER, ER-derived vesicles, and mitochondria surrounding LCV will decrease → Eventually gets replaced by ribosomes on LCV membrane
- LCV Remodeling: makes it ER-like to allow ribosome attraction
- *L. pneumophila* replication begins about 4-10 hours after phagocytosis

BACTERIAL MULTIPLICATION AND SPREAD

FORMATION OF THE LCV FOR REPLICATION: NUTRIENT ENVIRONMENT

- *L. pneumophila* recruit ubiquitinated proteins to LCV mediated by AnkB
- Ubiquitinated proteins are degraded
  - Process provides a source of amino acids for the replicating bacteria
- Amino Acid transporters are synthesized: “Phagosomal Transporters”
  - This is important for intracellular replication
- Iron acquisition
  - This is important for intracellular replication
- Autophagosomes may recruit to the LCV
  - Becomes a source of nutrients for replicating bacteria

BACTERIAL MULTIPLICATION AND SPREAD

FORMATION OF THE LCV FOR REPLICATION: ESCAPING THE LYSOSOME SUPPRESSING PATHWAY

- Bacteria replicate in vacuoles that would adopt a phagolysosome characteristic
- Bacteria in the later stages are more acid-resistant
- Recruitment of proteins involved in the endocytic pathway still takes place, except later than the usual cycle

BACTERIAL MULTIPLICATION AND SPREAD

FORMATION OF THE LCV FOR REPLICATION: TIME FOR SPREADING

- Amino acid starvation induces accumulation of ppGpp commences 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 of bacteria
- Cytotoxin production is induced
  - Can lead to host cell lysis

BACTERIAL MULTIPLICATION AND SPREAD

SECONDARY INFECTION

- If infection spreads outside of the lungs, they would be called secondary infections
  - SPLENOMEGALY: enlargement of the spleen and the rupturing of the spleen, pericarditis, wounds, joint infection, and CNS
- Hematogenous Spread
  - Would infect extrapulmonary sites, distributed via the bloodstream
- Infection could spread to other sites within the host through the macrophages they are replicating in, and once macrophage lyses occurs, bacteria gets spread into other potential infection sites

BACTERIAL DAMAGE

- Direct damage to the host
    - Bacterial replication will make host cell susceptible to lysis, and infection may spread to other host cells
    - Can eventually lead to host cell death as resources are exhausted
        - Via apoptosis and necrosis
    - Bacterial proteases can also lead to tissue damage within the host cell
    - Primary site of host cell damage: Pulmonary tissues and cells, including alveolar macrophages
    - Microbial components leading cell death have yet to be fully identified but the flagellin protein might be associated with the promotion of caspase 1-dependent cell death

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CONSEQUENTIAL SIGNS AND SYMPTOMS

- Production of interleukin-1 by monocytes
  - Responsible for signs and symptoms including fever response
- Recruitment of innate immune cells to the site of infection
  - Results in the increase of body temperature
  - Seen when Tom experienced a high fever on the cruise ship
- Other systemic immune responses could lead to other signs
  - Secretion of tumor necrosis factors
  - Headache
- Host can defend itself from the pathogen via physical clearance mechanisms
  - Damage or halting these mechanisms will result in an immunocompromised individual = more susceptible to diseases
  - Untreated Legionnaires can lead to serious complications like respiratory failure, septic shock, and multi-organ dysfunction

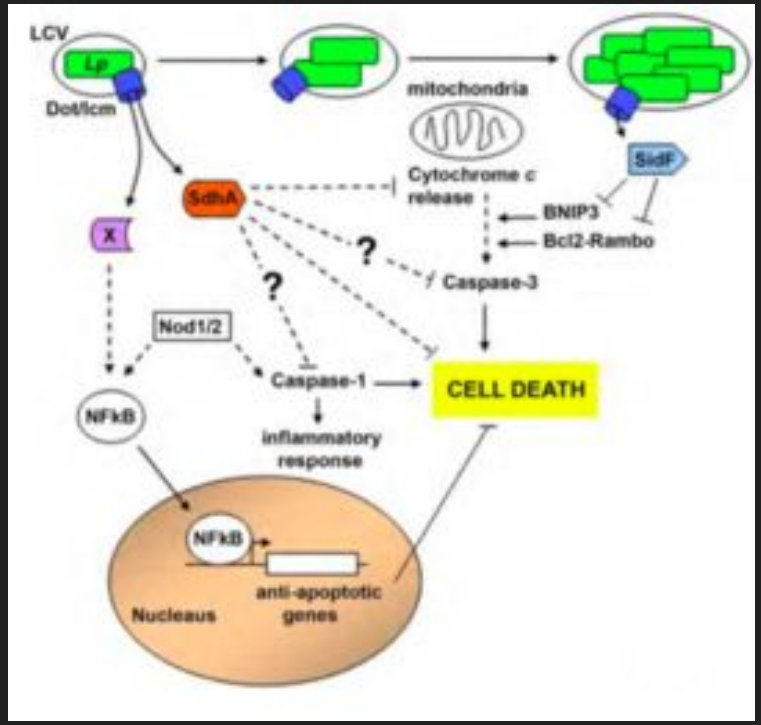
BACTERIAL DAMAGE

INDIRECT DAMAGE TO THE HOST

- Result of the host cell's defence response against the bacteria
- Neutrophils: Oxygen-dependent killing
  - Can lead to toxic concentrations of oxygen
  - Can lead to the accumulation of protein-rich fluid that floods the alveolar space
  - May result in the damage of the lung epithelial and endothelial cells
- Inflammatory Response: Recruitment of neutrophils and monocytes to the site of infection
- Leaky Capillaries: allow the influx of serum and increased deposition of fibrin in the alveoli

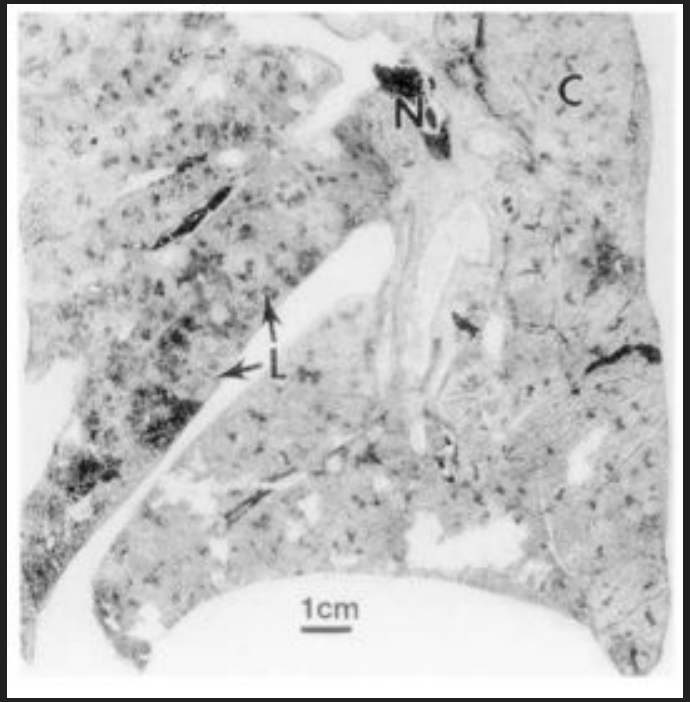
DEVELOPMENT OF PNEUMONIA

- Destroys the air spaces, disrupting the respiratory functions



MECHANISMS OF HOST CELL DEATH

- Mechanisms used by *L. Pneumophila*
- Host cell death and survival pathways



AIR SPACES

- Filled with fibrin and inflammatory cells