Lecture-22
Water Borne Human Pathogens

• Topics:
  • The genus *Vibrio*
  • *Vibrio cholerae*
    – In vivo life cycle of *V. cholerae*
      • Disease
      • Virulence factors
    – Evolution of pathogenic *V. cholerae*
  • *Legionella pneumophila*
    • Disease
    • Virulence factors

The genus *Vibrio*

• *Vibrios* are aquatic organisms
• They occur in both marine and fresh water habitats
• Have relatively simple growth factor requirements
  – grow in synthetic media with glucose as a carbon and energy source
  – require salt or sea-water based medium for optimal growth
  – tolerate alkaline media and sensitive to acid
  – Growth temperature varies 10-37C

The genus *Vibrio*

• Vary in metabolic versatility
  – Some species can grow on more than 150 different organic compounds as a carbon and energy source
  – Capable of both respiratory and fermentative metabolism
  – Some species can grow very fast (double in 10 minutes)
• Gram negative straight or curved rods
• Motile
  – In liquid media by means of single polar flagellum
  – On solid media they may synthesize lateral flagella
• Free living or in association with aquatic animals
  – Live in mutualistic association with fish and other marine life
  – Pathogenic to fish, vertebrates and invertebrates
Clinically important *Vibrio* species

- *V. vulnificus* and *V. parahaemolyticus*
  - Halophiles (require salt water environment)
  - Normal residents of coastal waters
  - Temperature and salinity of water influences their abundance (cell number increases during the summer)
  - In the US they are the most common vibrios associated with seafood borne illness
    - Oyster beds in Texas closed in the summer of 1998 (416 people in 13 states fell ill after eating oysters harvested from this location)
    - In the summer of 1997, *V. parahaemolyticus* caused large outbreak in the Pacific North West (209 cases, 1 death)

*Vibrio cholerae*

- Isolated in pure culture by Robert Koch in 1883
- Gram negative, facultative anaerobe
- Curved rod shaped
- Motile, single polar flagellum
- Inhabitants of brackish and estuarine waters
- Can grow in fresh and salt water
- Facultative human pathogen

Epidemiology of Cholera

- Cholera is endemic to India, Bangladesh, regions of South America, Africa, Australia and Gulf coast of the US
- There has been seven pandemics since 1817
  - First six started in Indian subcontinent (Ganges delta) and caused by Classical strains
  - The last one started in Indonesia and caused by El Tor strains.
Cholera

- Water-borne illness
- Transmitted by ingestion of food or water contaminated with *V. cholerae*
- The Infectious Dose- ID$_{50}$ varies depending on the pH of the stomach. In healthy volunteers $10^8$ bacteria produces infection, after neutralization of stomach acid $10^4$ bacteria can cause disease
- The small intestine is the primary site of infection
- *V. cholerae* colonize the epithelium without invasion or apparent damage; i.e., This is an extracellular pathogen that does not invade host tissue.
- Clinical symptoms are voluminous diarrhea and dehydration

Animal models

- **Human volunteers:** Since Cholera is only a disease of humans, this is the only true model. The Center for Vaccine Development (CVD) at the University of Maryland is the site for many human trials.

- **Rabbit ligated ileal loop:** Adult rabbit ileum is divided into 5-10-cm segments by suture and injected with live bacteria or test material. After 12-24h, animal is sacrificed; loop length and fluid volume measured as ml/cm readout. This assay is useful to quantitate toxin.

- **Infant mouse** (most common current model): Newborn mice are inoculated with bacteria; at sacrifice, stomach and entire gut are removed and weighed; gut wt/carcass wt = fluid accumulation ratio.

- Bacteria can be cultured and competitive index determined.
  - What are the pros and cons of using a competitive index?
**Virulence Factors**

**Discovery of Cholera Toxin (CT)**
- In 1959 De and Chatterjee injected living *V. cholerae* or cell free filtrates into the lumen of ligated rabbit ileal loop and observed large amount fluid accumulation.
- In 1969 Finkelstein et al. purified and characterized the toxin.
- In 1983, by administering purified CT to volunteers, Levin et al. were able to conclusively demonstrate that the toxin is the major mediator of the cholera syndrome.
- Ingestion of only 5 μg of purified toxin resulted in production of 1-6L of diarrheal stool.

**Cholera Toxin**
- **Structure**
  - It is composed of two polypeptides, A subunit and B subunit.
  - The ratio of B:A is 5:1
- The biological activity of CT is dependent on binding of the B pentamer to specific receptors on the eukaryotic cell.
- The B oligomer binds with high affinity exclusively to GM1 ganglioside.

**Pathogenesis: Mechanism of Action**
- Normally, the epithelial cells of the inner lining of the intestines (lumen) transfer sodium and chloride ions from the inside of the intestines to the blood stream.
- The "B" subunit of cholera toxin is bound by a host receptor (like a specific "landing pad") allowing the "A" subunit to enter the cell.
- Once inside the cell the "A" subunit causes a change in the regulation of the cells genes and as a result, the flow of ions and water is reversed.

**Cholera toxin**
- Mode of Action
  - Catalyzes an ADP-ribosylation reaction
  - CTX binds to surface sugar on the intestinal cells via B subunits
  - After binding A subunit which has ADP-ribosylating enzymatic activity is transported into host cell
  - Inside the cell, the A subunit ADP ribosylates (attaches an ADP-ribose) a G protein (largest family of mammalian cell-surface receptors, they mediate cellular response to signaling molecules)
  - G protein regulates activity of adenylate cyclase
  - ADP ribosylation leads to increased adenylate cyclase activity
  - Increased cAMP levels
  - Increased cAMP within intestinal epithelial cells leads to increased Cl- secretion
  - Osmotic imbalance causes water flow into the intestinal lumen—DIARRHEA—
Virulence Factors

- **Adherence**
  - Long filamentous pili termed the toxin-coregulated pilus (TCP) that form bundles on bacterial surface is essential for colonization
  - Mutants lacking TCP are avirulent in human volunteers and in animal models
  - Genes necessary for production of TCP are organized in an operon that contains 15 genes which is part of Vibrio pathogenicity island (VPI)
    - Major pilin subunit
    - Assembly
    - Secretion

Pathway that regulates expression of the major *V. cholerae* virulence factors CT and TCP

- **CTX genetic element**
  - Cholera toxin genes are encoded on a lysogenic filamentous bacteriophage
  - The phage can infect strains of *V. cholerae* missing toxin genes
  - The receptor for the phage is Toxin co-regulated pilus (TCP)
  - Transfer can occur in the gastrointestinal tract
  - Transfer can occur in the aquatic environment
**Treatment: Oral Rehydration Salts (ORS)**
- Reduces mortality from over 50% to less than 1%
- Packets of Oral Rehydration Salts
  - Distributed by WHO, UNICEF
  - Dissolve in 1 L water
  - NaCl, KCl, NaHCO₃, glucose

**Vaccine Development**
- A single clinical exposure to *V. cholerae* O1 confers protective immunity
- Live attenuated strain of *V. cholerae* as a vaccine
  - Ctx deletion strains still caused mild diarrhea
  - TCP mutants failed to induce significant protection
  - Vaccine trials have been unsuccessful in clinical trials
- Rehydration therapy remains essential in cholera treatment

**Vibrio cholerae**
- Inhabitant of aquatic ecosystems
- Free-living
- Biofilm state
  - phytoplankton, zooplankton, aquatic plants, insects, sand
- Removal of particles >20 μm from water reduced cholera incidence by 48%

**Legionnaires' Disease**
- caused by *Legionella pneumophilia*
  - gram-negative rod
  - Motile
  - Aerobic
- spread by airborne transmission from environmental reservoir to human host
  - soil, aquatic ecosystems, air-conditioning systems, and shower stalls
Legionnaires' disease

- reproduction of bacterium in alveolar macrophages causes localized tissue destruction

- Clinical manifestations
  - fever, cough, headache, neuralgia, and bronchopneumonia

- Treatment, prevention, and control
  - isolation of bacteria and immunodiagnostics
  - symptomatic/supportive therapy and antibiotic therapy
  - identification and elimination of environmental source

- Susceptibility
  - healthy are relatively resistant, impairment of respiratory defenses (heavy alcohol use, smoking, old age) increases susceptibility, hospital patients with underlying immune defects also susceptible

History

- The bacterium *Legionella pneumophila* was first identified in 1977, as the cause of an outbreak of severe pneumonia in a convention centre in the USA in 1976.

- *Legionnaire’s Disease* has been associated with outbreaks linked to poorly maintained artificial water systems, particularly cooling towers or evaporative condensers associated with air conditioning and industrial cooling, hot and cold water systems in public and private buildings, and whirlpool spas.


**History**

*Legionella* is a facultative human pathogen model for intracellular life style

- Legionella, are freshwater bacteria that are found in aquatic environments worldwide.
  - artificial water systems sometimes provide environments conducive to the growth of *Legionella* bacteria.

- These bacteria survive within or between the cells as parasites of free-living protozoa and within biofilms which develop in water systems where bacteria survive.

- Legionella live in phagocytic cells
15 min after phagocytosis

Small, smooth vesicles appear at the Legionella-containing vacuole (LCV), and appear to fuse with the phagosome membrane. Some mitochondria are near the LCV.

Horwitz, MA. JEM, 1983

1h after phagocytosis

In addition to smooth vesicles, mitochondria are recruited to the LCV and are intimately juxtaposed with the phagosome membrane.

4-8 hours after phagocytosis

completely surrounded by ribosome-studded rough ER.

Diagram of the sequence of cytoplasmic events involved in formation of the *L. pneumophila* phagosome
**Phagocytosis**

A macrophage engulfs the invading bacterium, and a membrane develops to form a phagosome.

**Virulence factors**

Interaction with macrophages.
- Outer membrane protein: Mip (macrophage invasion protein)
  - may promote invasion
  - mip mutants
    - reduced invasion of human macrophages in vitro
    - increased LD$_{50}$ for guinea pigs
    - no effect on growth of internalized bacteria

**Dot/Icm (defect in organelle trafficking/intracellular multiplication) genes are required for intracellular growth of Legionella**

- Many Gram-negative bacteria utilize a type IV secretion system (T4SS) to deliver proteins and DNA into a recipient cell.
- The Legionella pneumophila Dot/Icm T4SS is an essential virulence determinant that translocates effector proteins into eukaryotic cells.
- Effector proteins translocated by the Dot/Icm system function to delay endocytic maturation of the vacuole in which the bacterium resides, as well as promote remodeling of the L. pneumophila–containing vacuole into an organelle that resembles the endoplasmic reticulum.
- L. pneumophila replication occurs in the ER-derived vacuole created by these Dot/Icm-dependent processes.
- Currently, over 50 effector proteins have been shown to be translocated into eukaryotic cells by the Dot/Icm T4SS, and recent predictions suggest that there could be as many as 150 effectors.
Virulence factors

• Bacteria divide in vacuole, then escape to cytoplasm and grow until the cytoplasm is filled.
  – Effector proteins translocated by the Dot/Icm system is important.
  – Phospholipase C
    – hydrolyzes phosphatidylycholine
    – could allow escape from phagosome
• Bacteria lyse lung cells
• Extracellular proteases contribute to damage

Legionella infection cycle

Similarity of the mechanisms involved in the entry, traffic, replication, and exit of *L. pneumophila* with respect to both amoebae and macrophages

<table>
<thead>
<tr>
<th>Life cycle stage</th>
<th>Free-living amoebae</th>
<th>Macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entry</td>
<td>Coiling phagocytosis</td>
<td>Coiling phagocytosis</td>
</tr>
<tr>
<td>Traffic</td>
<td>No phagosome-lysosome fusion</td>
<td>No phagosome-lysosome fusion</td>
</tr>
<tr>
<td>Phagosome</td>
<td>Association with rough endoplasmic reticulum</td>
<td>Association with rough endoplasmic reticulum</td>
</tr>
<tr>
<td>Replication</td>
<td>Intraphagosomal</td>
<td>Intraphagosomal</td>
</tr>
<tr>
<td>Exit</td>
<td>Host cell lysis</td>
<td>Host cell lysis</td>
</tr>
</tbody>
</table>

Summary

• *Vibrio cholerae*
  – Acquired by ingestion of contaminated food or water
  – Facultative human pathogen, has an aquatic life cycle
  – Major pandemics throughout the history
  – Characterized by severe diarrhea
  – Major virulence factors are Tcp pili, ctx toxin.

• *Legionella pneumophila*
  – spread by airborne transmission from environmental reservoir to human host
  – Facultative human pathogen, has an aquatic life cycle
  – Major virulence factors are Type IV secretion system and effectors, Phospholipase C, Protein kinases Outer membrane protein: Mip.
Study Questions

1) What are the major virulence factors of *Vibrio cholerae*?

2) How is the cholera toxin discovered?

3) How do the pathogenic *Vibrio cholerae* strains evolve?

4) What are the major virulence factors of *Legionella pneumophila*?

5) What is unique about the transmission of waterborne pathogen *Legionella pneumophila*?

6) Although Legionella normally lives in water, it can only be cultured in the laboratory on a rich medium. Give possible explanation for this observation.