Pathogenic factors II: Toxins

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Pathogenesis of Bacterial Infections
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Goals for today:

1. Think about pathogenesis from a bacterial perspective
2. Discuss the role of toxins in promoting infection
3. Describe some strategies used by ‘major human pathogens’
4. Discuss any additional questions (throughout)
**Goals of a bacterial cell:**

1. Make more bacterial cells

How?
- Acquire nutrients
- Synthesize proteins & cellular structures
- Accurately replicate the genome
- Divide: partition into daughter cells
Goals of a **pathogenic** bacterial cell:

1. Make more bacterial cells

How?
- Acquire nutrients
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Goals of a pathogenic bacterial cell:

1. Make more bacterial cells

How?
• Acquire nutrients *Host can sequester these
• Synthesize proteins/cellular structures *Host can detect these
• Accurately replicate the genome *Host stressors can cause DNA damage, resulting in mutations
• Divide: partition into daughter cells *Can result in release of bacterial ligands that are sensed by the host

• Survival
  • Host is actively trying to eliminate bacteria
How do bacteria cause infection?

• Attachment—adherence to host surfaces (epithelium, tissues)
• Colonization—establishing a population at this site (replication)
• Avoiding the host response
• Dissemination—detachment, movement to the next site or host

**Toxins can aid in this process (really #3 and #4 above)**

**And toxins cause much of the disease pathology observed during infection**
What are toxins?

• Toxin: factor that promotes the death of another cell
  • Protein (many)
  • lipid (lipid A of LPS)
  • peptide (bacteriocin)
  • small RNA (toxin/anti-toxin)
  • Etc.
• Bacteria produce toxins that target other bacteria, and toxins that target host cells

Host cell-targeting:
• Endotoxin: inhibitory compound that remains bacterial cell-associated
  • LPS lipid A component: only associated with Gram-negative bacteria
• Exotoxin: secreted or released inhibitory compound
  • Superantigens (promote MHCII-TCR interactions that promote T cell proliferation and activation)
    • Enterotoxins
  • A/B toxins
  • Pore-forming toxins
LPS (endotoxin) structure and host sensing

A/B toxins

• A: effector domain with catalytic activity
  – ADP ribosylation, glucosylation, proteolysis
• B: receptor binding domain (defines tropism), translocates the A subunit
  – Can occur either at the plasma membrane or intracellularly
  – B subunits can be monomeric or oligomerize to form a pore
• Many examples:
  – Diphtheria, Cholera, Tetanus, Pertussis, Anthrax, Clostridia, Shiga, etc.

A/B toxins: Diphtheria toxin

Diphtheria Toxin: produced by Corynebacterium diphtheriae

- Gram-positive rod shaped bacterium
  - Club-shaped end, V-shaped morphology
- Very low incidence in the U.S. and Europe
  (high vaccine coverage: DTaP or Tdap)
  - Vaccine: diphtheria, tetanus, and pertussis
  - Inactivated toxoid components
- Respiratory infection
- Fibrinous pseudomembrane forms over tonsils, pharynx, larynx, also associated with bleeding
- Symptoms due to toxin activity causing host cell death
- Toxin expressed from a lysogenic phage
  - Common: also true of cholera & shiga toxins

Image: [www.cdc.gov](http://www.cdc.gov), Diphtheria photos
A/B toxins: Diphtheria toxin

Diptheria Toxin: produced by *Corynebacterium diphtheriae*

- Single polypeptide chain
- B subunit binds human heparin-binding EGF R
- Translocation occurs after trafficking into endosomes
  - pH dependent translocation
- A subunit ADP-ribosylates EF-2
  - Several other toxins target EF-2 (*Pseudomonas* exotoxin)
- Results in loss of translational elongation

**Used in mouse models to ablate specific cell types:**
- Express DTR on specific cells
- Treat with DT

A/B toxins: Cholera toxin

Cholera Toxin: produced by *Vibrio cholerae*

- Gram-negative, comma-shaped bacteria
- Intestinal extracellular pathogen
  - Forms biofilm structures on small intestinal epithelium
  - Also within aquatic environment
- $AB_5\ A/B$ toxin: single A subunit + 5 B subunits
- B subunits form a ring/pore
- Toxin expressed from a lysogenic phage
- Results in release of electrolytes into the intestinal lumen & characteristic diarrhea

A great book about a Cholera outbreak

Story of one of the first studies in epidemiology: 1854 Cholera outbreak in London

Dr. John Snow, Rev. Henry Whitehead, and the Broad Street pump
Pore-forming toxins (PFTs)

• Produced by Gram-negative and Gram-positive bacteria, almost all pathogens produce at least one
• Largest group: cholesterol-dependent cytolysins (CDCs)
  – Primarily produced by Gram-positive bacteria
  – *Pnemolysin, LLO, PFO, streptolysin O*

• Vary in number of subunits/pore size
• Cholesterol binding initiates monomeric insertion and forms a pre-pore complex
  • Pre-pore inserts
  • Oligomerization occurs within the membrane

Pneumolysin: produced by *Streptococcus pneumoniae*

*Streptococcus pneumoniae*: Gram-positive, extracellular pathogen
- Commensal of upper respiratory tract, can replicate here to cause pneumonia, septicemia, otitis media, and meningitis

- Targets neutrophils infiltrating into airway lumen
- Release of elastase from neutrophils can induce permeability in the epithelium & general tissue damage (impaired lung function)

Listeriolyasin O (LLO): produced by *Listeria monocytogenes*

*Listeria monocytogenes*: Gram-positive, intracellular pathogen
- Intestinal pathogen: Enters phagocytic and non-phagocytic cells

- Targets lysosomal membrane for escape into the host cytosol
- pH sensitivity: leads to pore formation specifically within endosomal compartments

Thinking about a toxin ‘repertoire’: *Staphylococcus aureus* toxins

*Staphylococcus aureus*: Gram-positive, extracellular pathogen
- Colonizes anterior nares, common member of skin microbiota
- Can infect any tissue: primarily causes respiratory or skin and soft tissue infections

- Multiple PFTs:
  - Alpha toxin: pore-forming toxin, binds ADAM10
  - Bicomponent leukocidins:
    - PVL (LukSF)
    - LukED
    - LukAB
  - Gamma toxin: HlgAB, HlgCB
- Phenol-soluble modulins (PSMs): secreted peptides, membrane disrupting
- Enterotoxins: produce about 20 (superantigens)

- Why so many???

Host cell defense: membrane repair mechanisms
Therapeutic approaches: to combat PFT activity

Why would you want to produce these?

- A/B toxins and Large PFTs: more likely to cause cell death, can promote dissemination
- Small PFTs: more likely to be sensed, and repaired

- Triggering cell death can allow bacteria to continue to replicate
  - Immune cells can be directly targeted

- Sensing by the host isn’t always a negative for bacteria
  - Recruited immune cells can provide nutrients (or cause damage that releases nutrients from other cells)
  - Intracellular pathogens need to replicate within host cells
  - Additional virulence factors can pacify immune cells

- Loss of toxin generally attenuates a pathogen: these are critical for replication within the host
Summary: Goals of a pathogenic bacterial cell

1. Make more bacterial cells

How?
• Acquire nutrients
• Synthesize proteins & cellular structures
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• **Survival**
  • Host is actively trying to eliminate bacteria