Pathogenesis of bacterial diseases – some specific examples

DONAL O’TOOLE
DEPT VET SCI
PATB 4130/5130

What pathogenic bacteria do to survive

- Enter host
- Evade:
  - Innate
  - Acquired immune system
- Adhere to host cells
- Acquire nutrients
- Escape host
- Survive extracellular environment

1. Lysis of inflammatory cells - pasteurellosis

Mannheimia haemolytica
- Esp. S1 serotype
- Important cause of pneumonia in cattle ('shipping fever') and other ruminant species
- Normal inhabitant of nasopharynx
- "Stress" = proliferation of S1 serotype → colonization of lower respiratory tract
- Disease: severe fibrinous pneumonia:
  - Massive influx of neutrophils and macrophages
  - Massive tissue damage in lung
Virulence factors in pasteurellosis

<table>
<thead>
<tr>
<th>Component</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesins</td>
<td>Adherence to respiratory epithelium</td>
</tr>
<tr>
<td>Capsule</td>
<td>Colonization of lung</td>
</tr>
<tr>
<td>LPS</td>
<td>Pro-inflammatory cytokines</td>
</tr>
<tr>
<td></td>
<td>Activation of complement and coagulation cascades</td>
</tr>
<tr>
<td>Outer membrane proteins</td>
<td>Iron acquisition</td>
</tr>
<tr>
<td>Leukotoxin</td>
<td>RTX-leukocyte cytolysis (specific for ruminant integrins on leukocytes)</td>
</tr>
<tr>
<td>Proteases</td>
<td>Neuraminidase: reduces surface mucus Immunoglobulin proteases</td>
</tr>
</tbody>
</table>
2. Potent exotoxins - anthrax

- *Bacillus anthracis*
- Gram positive spore-forming aerobic bacillus
- Evolved from *B. cereus* with acquisition of two plasmids and loss of some chromosomal genes
- Humans and herbivores susceptible; carnivores generally resistant
- Routes of exposure (incubation period days to weeks)
  - Cutaneous abrasions: resolve without complications in 80-90%
  - Ingestion: mortality rate 25% - 60%
  - Inhalation: mortality ~100%

Anthrax spores (malachite green stain)
Spores enable anthrax to remain in the environment for decades.
Spores are the major means of transmission.
Pathogenesis

Spores in environment
↓
Enter host (skin; digestive tract; lungs)
↓
Uptake by macrophages
↓
Escape from phagocytic vesicle
↓
Transported to LNs
↓
Formation of vegetative forms
↓
Toxin secretion
↓
Bacteremia/toxemia (10^7 to 10^8/ml)

Inhalation anthrax - mediastinitis

Virulence factors – *B. anthacis*

<table>
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<tr>
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<th>Activity</th>
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<tbody>
<tr>
<td>Spore formation</td>
<td>Protection in environment after O_3 exposure</td>
</tr>
<tr>
<td>Protective antigen (PA)</td>
<td>Binary component with LF or EF</td>
</tr>
<tr>
<td>Lethal factor (LF)</td>
<td>Inactivation of MAP kinase kinases</td>
</tr>
<tr>
<td>Edema factor (EF)</td>
<td>Oxidative burst; shock-inducing cytokines (TNFα and IL-1β)</td>
</tr>
</tbody>
</table>
3. Toxin secretion – *E. coli*

- **O157:H7**
- Enterohemorrhagic (EHEC) strain
- **Signs**
  - Watery or bloody diarrhea, nausea, cramps
  - Onset: 2-5 days
  - Duration: 5-10 days
- **Sequela**
  - Renal failure in 5-15% of patients
  - Diarrhea-associated* hemolytic uremic syndrome (HUS)
  - Thrombotic microangiopathy
  - Acute renal failure in children
  - Life threatening

*: other non-E. coli-associated forms of HUS exist
3. Hiding in cells: *Legionella pneumophila*

- Legionnaires’ disease
- Pneumonia
- Opportunistic/accidental pathogen
- Self-limiting infections (most)
- Disease when
  - Immunodeficiency
  - Poor respiratory defenses:
    - Smoking, COPD, age
  - Case fatality rates: 5 – 40%
  - Not natural pathogen in animals

**Virulence factors for *L. pneumophila***

- Environmental survival
  - Free living Gram-negative bacillus
  - Commensal in free-living, freshwater, and soil amoebae
    - Exposure to biocides, antibiotics, osmotic/thermal stress
    - Replication in protozoa predicts replication in alveolar macrophages
    - Acquisition of multiple eukaryotic genes
  - Two morphological forms:
    - Nutrient-rich conditions = replicative
    - Nutrient-limited conditions = transmissive (? virulence traits)
- Acquisition of eukaryotic genes as virulence factors
- Biofilms
  - More resistant to disinfectants
Intracellular uptake, survival and release

- Interaction with phagocytes
  - Uptake by conventional or coiling phagocytosis
  - No acidification → no phagolysosome fusion
  - Bacteria divide
  - Vesicle studded with ribosomes
  - Pore formation and lysis of vesicle
- Replicative → transmissive form
  - Motile
  - Altered cell envelope
  - Reduced or no cell division
  - More tolerant of environmental stress
- Controlling apoptosis:
  - No apoptosis early in infection
  - Apoptosis induced to release bacteria
  - Macrophages and pneumocytes → pneumonia

Induction of inflammasome

Pathogen → PRRs recognition → Caspase 1-oligomer activated → IL-1B and IL-18
4. Immune-mediated disease: streptococci

- *Streptococcus equi* subsp. *equi*
- Gram-positive aerobic bacterium
- Member of “pyogenic” streptococci
- Clone or biovar of *S. zooepidemicus*
- Host-adapted
- Cause of strangles in horses:
  - Acute respiratory disease ± complications
  - Carrier state in small proportion of recovered horses:
    - Guttural pouch or cranial sinuses
- Virulence largely determined by surface structures:
  - Hyaluronic acid
  - Anti-phagocytic M proteins

M and M-like proteins in streptococci

- Bind complement and complement regulatory proteins
- Highly variable
  - Vaccine use
- Antigenic cross-reactivity with host proteins:
  - Glomerulonephritis (multiple types)
  - Rheumatic fever (multiple types):
    - Cardiac myofiber myosin
    - Smooth muscle cells of arteries,
    - Perivascular connective tissue
Virulence factors of streptococci

<table>
<thead>
<tr>
<th>Virulence factor</th>
<th>Name</th>
<th>Specific effect</th>
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<tbody>
<tr>
<td>Adherence</td>
<td>Fibronectin-binding protein</td>
<td>Fibronectin</td>
</tr>
<tr>
<td></td>
<td>Fibrinogen-binding protein</td>
<td>Fibrin/fibrinogen</td>
</tr>
<tr>
<td></td>
<td>Hyaluronic acid</td>
<td>Early, non-specific binding</td>
</tr>
<tr>
<td></td>
<td>Lipoteichoic acid (LTA)</td>
<td></td>
</tr>
<tr>
<td>Immune evasion</td>
<td>M-like protein (S. mitis, S. mutans)</td>
<td>1. Inhibit C3b deposition; 2. Binding fibrinogen; 3. Keratinocytes</td>
</tr>
<tr>
<td></td>
<td>Superantigens (SPE, –)</td>
<td>T cell antigens → TNF and IL-1</td>
</tr>
<tr>
<td></td>
<td>SOD</td>
<td>Counteract neutrophil oxidative burst</td>
</tr>
<tr>
<td>Nutrient acquisition</td>
<td>Acid phosphatase</td>
<td>Lysis of cell surface lipoprotein</td>
</tr>
<tr>
<td></td>
<td>ATP-binding cassette (ABC)</td>
<td>Amino acids, metals, sugars</td>
</tr>
<tr>
<td></td>
<td>Degradative enzymes (streptolysin S-like toxin, hyaluronidase)</td>
<td>Cell lysis; destruction of GAG</td>
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Complications of strangles in horse

- Internal infection (bastard strangles)
  - Failure to localize in LNs
- Purpura hemorrhagica:
  - Immune complex deposition in vessels
  - Leukocytoclastic vasculitis
  - Can occur after vaccination with M-like protein
  - IgG/IgA ratio?
  - Other causes (in some - no obvious causes) also
- Myositis
  - Cross reaction between myosin and M-like protein
- Glomerulonephritis
  - Immune mediated

5. Moving cell-to-cell: Listeriosis

- *Listeria monocytogenes*
  - Other *Listeria* spp. cause abortions and septicemia in ruminants
- Gram-positive rod
- Facultative intracellular pathogen
- 13 serovars of *L. monocytogenes*
- Soil living + GIT carriage + poor (low pH) silage
- Humans: generally immunosuppressed or young/old
- Cattle: susceptibility factor not identified
Listeria monocytogenes

- Exemplary intracellular pathogen:
  - Macrophages and epithelial/neuronal cells
- Crosses three major barriers: intestine; BBB; placenta
- Diverse environments:
  - Wide temperature range (4 – 45°C)
  - Wide pH range (5 – 9)
  - Survives intestinal tract
- Important in cattle/sheep
  - Encephalitis
  - Abortion
- Important in people
  - Food poisoning/septicemia (rare)
  - Abortion, stillbirth, preterm labor, neonatal meningitis

Brainstem of a goat with listeriosis

Brainstem encephalitis

Microabscesses
Virulence factors

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<td>Adhesin (ActA)</td>
<td>Binds to heparin; initial attachment</td>
</tr>
<tr>
<td>Adhesin (InIA and B)</td>
<td>Binds to E-cadherin in enterocytes (A); endothelial cells and hepatocytes (B)</td>
</tr>
<tr>
<td>Listeriolysin</td>
<td>Pore-forming hemolysin – control in pH of cytoplasmic vacuole</td>
</tr>
<tr>
<td>Phospholipase C (a)</td>
<td>Degrade cytoplasmic membrane</td>
</tr>
<tr>
<td>ActA</td>
<td>Actin polymerization (actin tail)</td>
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<tr>
<td>Metalloprotease (Mpl)</td>
<td>Degrade cytoplasmic membrane</td>
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5. Biofilm formation: Pseudomonas

- Gram-negative nonsporulating rod
- Motile
- Related to select agents *Burkholderia* spp.
- Green pigments (pyocyanin; pyoverdin)
- Nutritional versatility
  - Multiple habitats (water, soil, plants, sewage)
  - Medical equipment and solutions
  - Opportunist infections
- Human:
  - Often nosocomial
  - Burns; cystic fibrosis; leukopenia; HIV/AIDS; neoplasm; surgical sites
- Animals:
  - Otitis externa, cystitis, keratitis, pneumonia, mastitis
- Resistance to many antibiotics
## Virulence factors - 1

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<tr>
<td>LasA and LasB elastase</td>
<td>Damage to pulmonary tissue and vessels</td>
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<td>Cytotoxins</td>
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<td>Ciliostatic</td>
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<tr>
<td>Phospholipase C</td>
<td>Destruction of surfactant</td>
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**P. aeruginosa**

**Biofilm formation in P. aeruginosa**

- 1 day in nutrient-poor medium
- 5 days in nutrient-poor medium
Virulence factors - 2

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P. aeruginosa + breach in innate/adaptive host defense

- Adherence/colonization
  - Attachment to host cells
  - Bacteria multiply and colonize altered cells
  - Anti-phagocytic LPS provide resistance to killing by serum

Acute infections

- Cell-to-cell signaling coordinate production of extracellular virulence factors
- Proteases + toxins lead to vascular injury, dissemination, organ failure

Chronic infections

- Alginate production
- Biofilm pattern of growth
- Reduced production of extracellular virulence factors
- Damage due to chronic inflammation
Take home

- Selected methods of disease induction by bacteria
  - Potent exotoxin secretion:
  - Induction of autoimmune disease:
  - Biofilm formation:
  - Migrating via nerves and cell-to-cell:
  - Direct destruction of leukocytes:
  - Intracellular survival: