Anaerobic infections

PART 1: Infection with Gram-positive obligate anaerobes (Invasive *Clostridium* spp.)

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What is an obligate anaerobe?

- **Obligate aerobes**
  - acquire energy *ONLY* by respiration
  - cannot survive without oxygen
    - ex. *Mycobacterium tuberculosis, Pseudomonas aeruginosa*

- **Obligate anaerobes**
  - acquire energy *ONLY* by fermentation
  - most cannot survive in oxygen
    - ex. *Clostridium perfringens, Bacteroides fragilis*

- **Facultative anaerobes (most bacteria)**
  - acquire energy by either *respiration* OR fermentation
  - Can survive with or without oxygen
    - ex. *E. coli, Staphylococcus aureus*
Where do obligate anaerobes live?

- **Endogenous (in the body)**
  - GI tract of animals
  - Gingival crevices around teeth
  - Skin glands and hair follicles
- **Exogenous (in the environment)**
  - Soil
The Endogenous Microbiome

- Our bacteria > our own cells
- Roles of endogenous microbiota (microflora)
  - Digestion
  - Colonization resistance
  - Induction of antibodies (IgA, IgG, etc.)
  - Normal development of the immune response
- Should the microbiome be considered another organ of the body?
Sources of Anaerobic Infections

• Usually endogenous
  – Intestinal anaerobes
  – Oral anaerobes

• Usually exogenous
  – *Clostridium tetani* (tetanus)
  – *Clostridium botulinum* (botulism)
  – *Clostridium difficile* (antibiotic-associated colitis)

• Either endogenous or exogenous
  – Other Clostridial infections (e.g., gas gangrene)
What are these lectures about?

• **Part 1: Invasive Clostridium spp.**
  - gas gangrene/myonecrosis
  - wound infection/abscess
  - food poisoning
  - C. perfringens, C. septicum, C. histolyticum, C. novyi, etc.

• **Part 2: Toxigenic Clostridium spp.**
  - tetanus
  - botulism
  - antibiotic-associated colitis
  - C. tetani, C. botulinum, C. difficile

• **Part 3: Gram-negative anaerobes**
  - abscesses
  - other
  - B. fragilis, Bacteroides spp, Prevotella, Porphyromonas, Fusobacterium, anaerobic cocci
Case: infected wound

- 46 year old male construction supervisor fell ~4 meters onto an iron reinforcing rod at a construction site. The rod penetrated the left upper arm and tore the skin.
- The patient was told that he was diabetic many years earlier but was on no treatment.
- At the ED, the wound was sutured closed and bandaged. A pain killer and an oral antibiotic were prescribed. The patient took the pain killer only.
Case (continued)

• He returned 48 hours later with *extreme pain and tenderness* of the entire left arm
• Temperature=39.5C *(fever)*.
• *Thin watery drainage* from the wound.
• “Crunchy” sensation under the affected skin (i.e., *crepitus*).
• An xray of the shoulder shows *gas in the tissues* under the affected skin.
• Routine bacterial culture: negative.
Gram-stain of watery discharge

No intact PMNs were seen
Case (continued)

- The patient was hospitalized urgently and treated with high-dose penicillin
- He underwent several extensive debridements of devitalized muscle and skin, followed by muscle flap and skin graft closure.
- Anaerobic culture at his first surgery grew a Gram-positive rod with central spores.
Questions to consider?

- Where did these bacteria come from? Why were they at a construction site?
- Why are there no intact PMNs in the pus?
- Why is there gas in the tissue?
- Why was the routine culture negative?
- Why is this infection so aggressive in this patient?
- Why was it necessary to do repeated surgery if antibiotics are active?
**Clostridium** species

- Gram-positive, spore-forming bacilli
- Sporulation occurs in nutrient-limiting conditions
- Spores are resistant to extremes of dryness, heat (boiling), and many chemical disinfectants
- They can persist in the environment for weeks to months and germinate deep in the soil where the conditions are anaerobic
- In favorable conditions (e.g., in human tissues), spores germinate and may produce toxins
Sporulation

- Complex sequence of gene expression is triggered
- Asymmetric cell division, with partitioning of a chromosome into an internal spore (endospore)
- Elaboration of a thick, impermeable surface coat
Spores

Cell division and partitioning
Elaboration of a spore coat
Free spore

Spore positioning and species

- Terminal spore: C. tetani
- Central spore: C. perfringens
- Subterminal spore: C. septicum, novyi, histolyticum, difficile, botulinum
Wounds and soft tissue infection

- *C. perfringens* type A is the most common invasive clostridial species (but other species can cause similar effects – *C. novyi, C. septicum*)
- The organism requires damaged tissue with anaerobic conditions, impaired blood supply, complex nutrients, and Ca^{++} ions
- Produces 12 toxins that attack membranes, including:
  - α-toxin (lecithinase, AKA “myotoxin”)
  - zinc metallophospholipase (hydrolyzes phosphatidylcholine and sphingomyelin-kills cells)
- Toxins destroy PMNs, produce myonecrosis
- Organisms produce gas when they grow = crepitance in tissue
Management of gas gangrene

- Surgical debridement of all devitalized tissues (remove the anaerobic/necrotic focus)
- Penicillin and other beta-lactam antibiotics are effective but not sufficient
- Antitoxins are not effective
- Hyperbaric oxygen may be useful
- There is no vaccine
Clostridia may also be involved in milder non-necrotizing wound infections, often in combination with other pathogens, e.g., diabetic foot infections, leprous wounds.
Laboratory diagnosis

- To cultivate anaerobes, the specimen should not be exposed to air
Anaerobic growth

• Anaerobes will grow at the bottom of tubes of static nutrient broth
• To separate facultative from obligate anaerobes you must plate out the growth from the bottom of the tubes
Thought experiment

- *Pseudomonas aeruginosa*
- *S. aureus*
- *Clostridium spp.*

Soft agar tubes

37°C for 24 hours
Culturing anaerobes on plates

- Media (blood agar) should be pre-reduced
- Swabs should be pre-reduced when used
- Plating and culture is ideally done in an anaerobic environment
Another thought experiment

Mixture of: *Pseudomonas aeruginosa*
*S. aureus*
*Clostridium sporogenes*

37°C x 24h in air

37°C x 24h in an anaerobic chamber
Most obligate anaerobes produce a distinctly unpleasant odor!!
Questions to consider?

- Where did these bacteria come from? Why were they at a construction site?
- Why are there no intact PMNs in the pus?
- Why is there gas in the tissue?
- Why was the routine culture negative?
- Why is this infection so aggressive in this patient?
- Why was it necessary to do repeated surgery if antibiotics are active?
Case: Spontaneous gas gangrene

- A 50 year old man developed gas gangrene of his right shoulder without any predisposing trauma.
- Culture of the debrided tissues grew *C. septicum*
- Two months later, colon cancer was diagnosed.
Spontaneous *C. septicum* gangrene
Clostridial diseases in which culture is not usually done

- Food poisoning
- Tetanus
- Botulism
- Antibiotic-associated colitis
C. perfringens food poisoning

• Improperly-handled food is contaminated with spores, which survive cooking temperature
• Spores germinate with heating (anaerobiasis) and if >10^5/g bacteria are ingested, illness may occur
• Sporulation in the small intestine releases enterotoxin
• Diarrhea (without fever) occurs 6-18 hours later, and resolves in 1-2 days
Clostridial food poisoning

Food held too long at room temperature; cools slowly; spores germinate

Spores (Meat or poultry)

Bacteria sporulate in the small intestine; release enterotoxin
Diagnosis Management of Clostridial Food Poisoning

- Usually recognized by multiple cases of diarrhea 6-18 hours after ingestion of food (usually in restaurants, not home)
- Culture of the patient is not helpful
- Treatment is supportive only (resolves spontaneously); no antibiotics
- Identify the food vehicle, (culture), and correct preparation problem
Generalizations about invasive Clostridium spp.

- Sporulation is critical for survival in the environment (soil)
- Exogenous infections = spores in wounds; Endogenous infections = vegetative bacteria released from colonized sites (e.g., colon)
- Disease is mediated by exotoxin-release from vegetative cells
- Simple antibiotics are effective, but not in non-viable tissues; surgery is often required
- Antibiotic resistance is not a problem
Summary of Key Points

• *Clostridium* spp. are Gram-positive spore-forming, obligate anaerobic bacteria that grow in devitalized tissues.
• Alpha-toxin is a lecithinase that destroy cell membranes, including PMNs and muscle cells.
• Gas gangrene and myonecrosis may result from wound infection with certain clostridia.
• Antibiotics and surgery are critical if the patient is to survive.
• Clostridia may contribute to lesser, mixed bacterial wound infections.
• *C. septicum* bacteremia may signal colon cancer.
• Clostridial enterotoxin causes a self-limited diarrhea (food poisoning) but is not invasive most cases.
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