Anaerobes

Michael Yin, MD MS

Definitions

• Anaerobes
  – Bacteria that require anaerobic conditions to initiate and sustain growth
  • Ability to live in oxygen environment (detoxify superoxide ion)
  • Ability to utilize oxygen for energy instead of fermentation or anaerobic respiration

• Strict (obligate) anaerobe
  – Unable to grow if > than 0.5% oxygen

• Moderate anaerobes
  – Capable of growing between 2-8% oxygen

• Microaerophillic bacteria
  – Grows in presence of oxygen, but better in anaerobic conditions

• Facultative bacteria (facultative anaerobes)
  – Grows both in presence and absence of oxygen

Classification of Medically Important Anaerobes

• Gram positive cocci
  – Peptostreptococcus

• Gram negative cocci
  – Veillonella

• Gram positive bacilli
  – Clostridium perfringens, tetani, botulinum, difficile
  – Propionibacterium
  – Actinomyces
  – Lactobacillus
  – Mobiluncus

• Gram negative bacilli
  – Bacteroides fragilis, thetaiotaomicron
  – Fusobacterium
  – Prevotella
  – Porphyromonas

Epidemiology

• Endogenous infections
  – Indigenous microflora
    • Skin: Propionibacterium, Peptostreptococcus
    • Upper respiratory: Propionibacterium
    • Mouth: Fusobacterium, Actinomyces
    • Intestines: Clostridium, Bacteroides, Fusobacterium
    • Vagina: Lactobacillus
  – Flora can be profoundly modified to favor anaerobes
    • Medications: antibiotics, antacids, bowel motility agents
    • Surgery (blind loops)
    • Cancers

• Exogenous infections
  – Spore forming organisms in soil, water, sewage

Role of Anaerobes

• Prevent colonization & infection by pathogens
  • Bacterial interference through elaboration of toxic metabolites, low pH, depletion of nutrients
  • Interference with adhesion

• Contributes to host physiology
  • Bacteroides fragilis synthesizes vitamin K and deconjugates bile acids
Clinical features of anaerobic infections

- The source of infecting micro-organism is the endogenous flora of host
- Alterations of host’s tissues provide suitable conditions for development of opportunist anaerobic infections
- Anaerobic infections are generally polymicrobial
- Abscess formation
- Exotoxin formation

Sites of anaerobic infections

Virulence factors

- Attachement and adhesion
  - Polysaccharide capsules and pili
- Invasion
  - Aerotolerance
- Establishment of infection
  - Polysaccharide capsule (B. fragilis) resists opsonization and phagocytosis
  - Synergize with aerobes
  - Spore formation (Clostridium)
- Tissue damage
  - Elaboration of enzymes, toxins

Anaerobic cocci

- Epidemiology
  - Normal flora of skin, mouth, intestinal and genitourinary tracts
- Pathogenesis
  - Virulence factors not as well characterized
  - Opportunistic pathogens, often involved in polymicrobial infections
  - Brain abscesses, periodontal disease, pneumonias, skin and soft tissue infections, intra-abdominal infections
- Peptostreptococcus
  - P. magnus: chronic bone and joint infections, especially prosthetic joints
  - P. prevotii and P. anaerobius: female genital tract and intra-abdominal infections
- Veillonella
  - Normal oral flora; isolated from infected human bites

Anaerobic gram positive bacilli

- No Spore Formation
  - Propionibacterium
    - P. acnes
  - Actinomyces
  - Lactobacillus
  - Mobiluncus

- Spore Formation
  - Clostridium
    - C. perfringens
    - C. difficile
    - C. tetani
    - C. botulinum

TABLE 20-2 Conditions Predisposing to Anaerobic Infection

| General           | Diabetics | Carbohydrates | Lactobacteria | H. pylori | Immunosuppressive | Cytoskeletal drugs | Sphingomyelin | Obligate anaerobic | Other | Tissue necrosis and infarct | Tissue anaerobiosis | Tissue destruction | Aerobic infection | Paronychia | Urethritis | Cellulitis | Septicemia | Posterior | Hematologic | Specialized structures | Cervix | Colon, cecum, lung | Osteomyelitis | Gastrointestinal and female pelvic surgery | Gastrointestinal trauma | Human and extracellular | Anthrax proliferative therapy |
### Propionibacterium
- Produces propionic acid as major byproduct of fermentation
- Colonize skin, conjunctiva, external ear, oropharynx, female GU tract
  - *P. acnes*
    - Acne
      - Resides in sebaceous follicles, releases LMW peptide, stimulates an inflammatory response
    - Opportunistic infections
      - Prosthetic devices (heart valves, ventricular shunts)

### Pilosebaceous Follicle

### Actinomyces
- Facultative or strict anaerobe
- Colonize upper respiratory tract, GI, female GU tract
- Actinomycosis
  - Endogenous disease, no person-person spread
  - Low virulence; development of disease when normal mucosal barriers are disrupted (dental procedure)
  - Diagnosis made by examination of infected fluid:
    - Macroscopic colonies of organisms resembling grains of sand (sulfur granules)
    - Culture

### Actinomycosis
- Cervicofacial
- Poor oral hygiene, oral trauma, invasive dental procedure
- Chronic granulomatous lesions that become suppurative and form sinus tracts
- Slowly evolving, painless process
- Treatment: surgical debridement and prolonged penicillin

### Lactobacillus
- Facultative or strict anaerobes
- Colonize GI and GU tract
  - Vagina heavily colonized (10⁵/ml) by Lactobacillus crispatus & jensonii
  - Certain strains produces H₂O₂ which is bactericidal to Gardnerella vaginalis
- Clinical disease
  - Transient bacteremia from GU source
  - Bacteremia in immunocompromized host
  - Endocarditis

### Mobiluncus
- Obligate anaerobes
- Gram variable
- Colonize GU tract in low numbers
- Associated with bacterial vaginosis
  - Detected in vagina of 6% of controls
  - As many as 97% of women with bacterial vaginosis
Case 1

- 12 year old boy with Acute Myelogenous Leukemia (AML) diagnosed 2 mo. ago
- Pancytopenia after receiving chemotherapy
- Presented with painful ecchymotic areas on legs that rapidly progressed with marked swelling and pain over several hours
  - Afebrile
  - Crepitus in both legs
  - Rapid progression to shock

Case 1

- Needle aspirate of ecchymotic area revealed gram-positive bacilli
- Blood cultures grew Clostridium perfringens

Clostridium

- Epidemiology
  - Ubiquitous
  - Present in soil, water, sewage
  - Normal flora in GI tracts of animals and humans
- Pathogenesis
  - Spore formation
    - Resistant to heat, desiccation, and disinfectants
    - Can survive for years in adverse environments
  - Rapid growth in oxygen deprived, nutritionally enriched environment
  - Toxin elaboration (histolytic toxins, enterotoxins, neurotoxins)

Clostridium perfringens

- Epidemiology
  - GI tract of humans and animals
  - Type A responsible for most human infections, is widely distributed in soil and water contaminated with feces
  - Type B-E do not survive in soil but colonize the intestinal tracts of animals and occasionally humans
- Pathogenesis
  - α-toxin: lecithinase (phospholipase C) that lyses erythrocytes, platelets and endothelial cells resulting in increased vascular permeability and hemolysis
  - β-toxin: necrotizing activity
  - Enterotoxin: binds to brush borders and disrupts small intestinal transport resulting in increased membrane permeability
- Clinical manifestations
  - Soft limited gastroenteritis
  - Soft tissue infections: cellulitis, fasciitis or myonecrosis (gas gangrene)
Clostridial soft tissue infections

Crepitant cellulitis

Fascitis

Myonecrosis

- Clinical course
  - Symptoms begin 1-4 days after inoculation and progresses rapidly to extensive muscle necrosis and shock
  - Local area with marked pain, swelling, serosanguinous discharge, bullae, slight crepitance
  - May be associated with increased CPK
- Treatment
  - Surgical debridement
  - Antibiotics
  - Hyperbaric oxygen

Case 2

- 80 year old woman who was treated for a pneumonia with a cephalosporin
  - Well upon discharge from hospital
  - 10 days later develops multiple, watery loose stools and abdominal cramps
  - Fever, bloody stools, worsened abdominal pain

Clostridial myonecrosis

- Leukocytosis with 80% neutrophils
- Fecal leukocytes
- Stool culture neg. for salmonella, shigella campylobacter, Yersinia spp
- Colonoscopy
  - White plaques of fibrin, mucous and inflammatory cells

Clostridium difficile

- Epidemiology
  - Endogenous infection
  - Colonizes GI tract in 5% healthy individuals
  - Antibiotic exposure associated with overgrowth of C. difficile
    - Cephalosporins, clindamycin, ampicillin/amoxicillin
  - Other contributing factors: agents altering GI motility, surgery, age, underlying illness
  - Exogenous infection
    - Spores detected in hospital rooms of infected patients
- Pathogenesis
  - Enterotoxin (toxin A)
    - Produces chemotaxis, induces cytokine production and hypersecretion of fluid, development of hemorrhagic necrosis
  - Cytotoxin (toxin B)
    - Induces polymerization of actin with loss of cellular cytoskeleton
**C. difficile colitis**

- Clinical syndromes
  - Asymptomatic colonization
  - Antibiotic-associated diarrhea
  - Pseudomembranous colitis
- Diagnosis
  - Isolation of toxin
- Culture
- Treatment
  - Discontinue antibiotics
  - Metronidazole or oral vancomycin
  - Pooled human IVIG for severe disease
  - Probiotics (saccharomyces boulardii)
  - New drugs (nitazoxanide, tolevamer)
  - Relapse in 20-30% (spores are resistant)

**North American PFGE type 1 (NAP-1)**

- Epidemiology:
  - Quebec 2003: 56.3/100,000; 18% severe, 14% died within 30 days
- Pathogenesis
  - Produces greater quantities of toxins A and B *in vitro*
  - Deletion in the tcdC gene (a putative negative regulator of toxin production)
  - Contains a binary toxin
  - Selected by fluoroquinolone use

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**Clostridium tetani**

- Epidemiology
  - Spores found in most soils, GI tracts of animals
  - Disease in un-vaccinated or inadequately immunized
  - Disease does not induce immunity
- Pathogenesis
  - Spore inoculated into wound
  - Tetanospasmin
    - Heat-labile neurotoxin
    - Retrograde axonal transport to CNS
    - Blocks release of inhibitory neurotransmitters (e.g., GABA) into synapses, allowing excitatory synapses to be unregulated. This results in muscle spasms
    - Binding is irreversible
  - Tetanolysin
    - Oxygen labile hemolysin, unclear clinical significance

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**C. tetani exotoxin**

**Tetanus**

- Clinical Manifestations
  - Generalized
    - Involvement of bulbar and paraspinal muscles
    - Trismus (lock jaw), risus sardonicus, opisthotonos
    - Autonomic involvement
      - Sweating, hyperthermia, cardiac arrhythmias, labile blood pressure
      - Cephalic
        - Involvement of cranial nerves only
        - Localized
          - Involvement of muscles in primary area of injury
        - Neonatal
          - Generalized in neonates; infected umbilical stump
Risus sardonicus and Opisthotonos of Tetanus

Tetanus

- Treatment
  - Debridement of wound
  - Metronidazole
  - Tetanus immunoglobulin
  - Vaccination with tetanus toxoid
- Prevention
  - Vaccination with a series of 3 tetanus toxoid
  - Booster dose every 10 years

Case 3

- 6 month old infant girl, full-term, previously healthy
- Progressive fussiness, poor oral intake, weak cry for 4 days.
- Uninterested in feeding or playing.
- Exam:
  - Listless
  - Afebrile, stable vital signs
  - Sluggish pupils, decreased tone, no reflexes bilaterally
- No ill contacts or recent travel, lives with parents on Staten Island
  - Construction in neighborhood
- Diet: Breast milk & some rice cereal only
- No fever, vomiting, diarrhea, rash, seizures

Case 3

- Serum, breast milk, stool sent to DOH for detection of Botulinum toxin
  - Stool POSTIVE for toxin type B
- Given Baby botulism immunoglobulin (Baby-BIG)
  - Regained movement of arm within a day
  - Began feeding in 4 days
- Clostridium botulinum
  - Epidemiology
    - Commonly isolated in soil and water
      - 20% soil samples
    - Human disease associated with botulinum toxin A, B, E, F
  - Pathogenesis
    - Blocks neurotransmission at peripheral cholinergic synapses
    - Prevents release of acetylcholine, resulting in muscle relaxation
    - Recovery depends upon regeneration of nerve endings
C. Botulinum Exotoxin

Botulinism

- Clinical Syndromes
  - Foodborne botulism
    - Associated with consumption of preformed toxin
      - Home-canned foods (toxin A, B)
      - Preserved fish (toxin E)
  - Infant botulism
    - Consumption of foods contaminated with botulinum spores
      - 6-10% of syrups or honeys
      - Disease associated with neurotoxin produced in vivo
  - Onset of symptoms 1-2 days
    - Blurred vision, dilated pupils, dry mouth, constipation
    - Bilateral descending weakness of peripheral muscles; death related to respiratory failure

- Infant botulism
  - Onset of symptoms in 3-10 days

- Wound botulism (skin popping)
  - Asymptomatic adult carriage

Cases of Infant Botulism 1976-1996

Botulism: diagnosis

- Clinical features:
  - Symmetric cranial nerve palsies (III, IV, VI, VII) causing 4Ds: diplopia, dysphonia, dysarthria, and dysphagia
  - Symmetric flaccid paralysis
  - Mentation remains intact
  - Identification of toxin or organism in stool or serum
    - Mouse bioassay most sensitive
  - Electromyography

Botulism: Treatment

- Treatment
  - Supportive care
  - Elimination of organism from GI tract
    - Gastric lavage
    - Metronidazole or penicillin
    - Botulinum Immunoglobulin (BIG): pooled plasma from adults immunized with pentavalent (ABCDE) botulinum toxoid
    - Trivalent equine Immunoglobulin (ABE)
  - Prevention
    - Prevention of spore germination (Storage <4°C, high sugar content, acid pH)
    - Destruction of preformed toxin (20 min at 80°C)

Anaerobic gram negative bacilli

- Bacteroides
  - B. fragilis
  - B. thetaiotaomicron
- Fusobacterium
- Prevotella
- Porphyromonas
Anaerobic gram negative bacilli

- **Epidemiology**
  - Bacteroides and Prevotella are most prevalent organisms in human flora
  - Oral cavity (crypts of tonsils and tongue, dental plaques and gingival crevices)
  - Anaerobes become prominent after eruption of teeth
  - Porphyromonas gingivalis found in 37% of subjects, colonization concordance in families
  - Fecobacterium
    - GI tract
      - Anaerobes outnumber aerobes 1000:1
      - 10^11 organisms per gram of fecal material
  - Bacteroides spp. (vulgatus and thetaiotaomicron most common)
  - Vagina

- **Clinical Diseases**
  - Chronic sinus infections
  - Periodontal infections
  - Brain abscess
  - Intra-abdominal infection
  - Gynecological infection
  - Diabetic and decubitus ulcers

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**Case 4**

- 37 year old woman with peri-umbilical pain, anorexia, and nausea
  - Given diagnosis of food poisoning in the ER and sent home
  - Develops sharp right lower abdominal pain and fever over next 4 days

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**Bacteroides**

- **Epidemiology**
  - B. fragilis associated with 80% of intra-abd infx
  - Peritonitis, intraabdominal abscesses
  - Diabetic foot ulcers

- **Pathogenesis**
  - Polysaccharide capsule
    - Increases adhesion to peritoneal surfaces (along with fimbriae)
    - Protection against phagocytosis
    - Differs from LPS of aerobic GNR
    - Less fatty acids linked to Lipid A component
    - Less pyrogenic activity
  - Abscess Formation
    - Produces superoxide dismutase and catalase
    - Elaborate a variety of enzymes
    - Synergistic infections with aerobes

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**Abscess Formation**

- **Bacteroides Capsular Polysaccharide Complex (CPC)**
  - 2 discreet polysaccharides (PS A & PS B) with oppositely charged structural groups
  - Injection of CPC into peritoneum of rat results in abscess formation
    - Chemical neutralization or removal of charged groups abrogated abscess induction
  - Vaccination with CPC results in protection against abscess formation
    - T cells important in abscess formation

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Wainstein, Infection and Immunity, 1974

![Diagram](image-url)
Abscess Formation

- Initial phase
  - Introduction of bacteria and inflammatory exudates (esp. fibrin)
- Microbial persistence (localization)
  - Impaired bacterial clearance: fibrin deposition, platelet clumping
  - Impaired phagocytic function: fibrin, hemoglobin
  - Impaired neutrophil migration and killing: hypoxia, low PH
  - Complement depletion: necrotic debris
- Development of mature abscess
  - Central core of necrotic debris, dead cells, bacteria
  - Surrounded by neutrophils and macrophages
  - Peripheral ring of fibroblasts and smooth muscle cells within collagen capsule

Conclusion

- Anaerobic infections
  - Endogenous or exogenous
  - Alteration of host tissue
    - Break in anatomic barrier
    - Devitalized tissue
  - Polymicrobial
    - Synergy between anaerobes and facultative bacteria
  - Abscess formation
  - Exotoxin elaboration