Unit Lecture 11

Gram-positive Bacilli

Gram positive bacilli produce some of the most important and deadly toxins known to man. *Bacillus* and *Clostridium* species also are the only pathogens that produce endospores.

Bacillus anthracis is a large gram-positive single rod or in pairs *in vivo* but forms chains when grown in culture. They are nonmotile (rare for the genus), produce a polypeptide capsule and a central spore. Spores can survive in the soil for years. *B. anthracis* is a strict aerobe that is easily cultured on most common media. *Bacillus anthracis* is the causative agent of anthrax. The primary infection is variable based on POE. Cutaneous anthrax (Woolsorter's disease) occurs via breaks in skin. Pulmonary anthrax is a result of infection via the respiratory tract. Intestinal anthrax is acquired via the digestive tract. Cutaneous lesions become black and necrotic. Secondary infections involve the lymphatic system if the primary infection is untreated. Septicemia is fatal in 5-6 hours. Transmission and infection occurs by coming into contact with vegetative or spore forms (sources: soil, leather, wool, dead animals etc.). Edema toxin and lethal toxin are two virulence factors that cause the clinical manifestations. Treatment is with penicillin G and tetracycline but must occur early before toxin development. A vaccine was developed by Pasteur for animals.

Clostridium tetani is a gram-positive bacilli that forms short chains, has peritrichous flagella, and a terminal spore at end of the bacillus (tennis racquet). The organism is a strict anaerobe. *Clostridium tetani* causes tetanus. Infection is acquired via soil-contaminated wounds. Untreated tetanus causes tetani of skeletal muscles, usually the head and neck first, producing the symptoms of lockjaw (Risus sardonicus) and Opisthotonus. Death occurs from respiratory paralysis. Two forms of the disease are present. The first is one of short incubation (3 days - 2 weeks) and large numbers of organisms. It has a high fatality rate. The second has a long incubation (4-5 weeks). Fewer organisms are present initially. It produces a lower fatality rate especially if treated. The organism produces a potent neurotoxin, tetanospasmin, which is the second most powerful toxin known to man. The mechanism of infection involves a deep puncture wound that can sometimes occur with accident victims. It can also be the result of cultural practices and cause "tetanus neonatorum" which is the result of purposeful contamination of the umbilicus at birth with animal dung. Transmission is not communicable from man to man. Treatment involves the use of penicillin and tetracycline for the organism and antitoxin to neutralize the toxin. Sometimes hyperbaric chambers are also used to force oxygen into the tissues. Prevention of toxin

formation by toxin neutralization antibodies is achieved by vaccination with DPT and periodic prophylactic boosters.

Clostridium perfringens is a gram positive single, thick rod or pair of rods when *in vivo* but chains of rods in culture. It is nonmotile (rare for genus), produces a central to sub terminal spores, and is strictly anaerobic. It causes gas gangrene (anaerobic cellulitis or myonecrosis). It is an infection of anaerobic sites, a region of impaired blood supply (e.g. diabetes) or from deep, soil-contaminated wounds. Necrotic tissues and abscesses are produced by variety of toxins (necrotoxins, hemolysins, hyaluronidase, collagenase, and proteolysins) and virulence factors. The result is frothing and bubbling exudates, emphysematous (air-filled) tissues, and nauseating odors. Transmission is not communicable. Treatment involves tetracycline but is not often effective because of poor blood supply. Debridement of diseased tissue is necessary to remove substrate conducive to growth. Amputation and hyperbaric chamber are sometimes necessary. Antitoxins are only moderately helpful. Vaccines are not available and doubtful. This organism also causes food poisoning due to intoxication of preformed toxin.

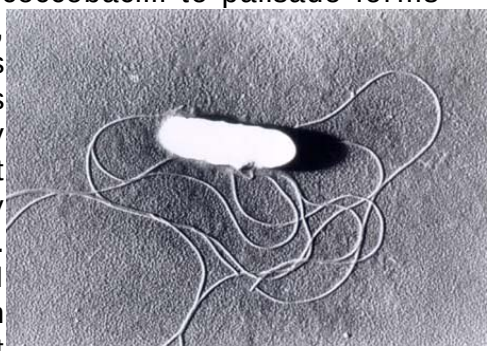
Clostridium botulinum is a gram-positive single rod, sometimes found in pairs, possessing peritrichous flagella, and subterminal oval spores. The organism, like other clostridia is strictly anaerobic. Botulism is an intoxication disease resulting usually from the ingestion of preformed toxins. Neurotoxins block acetylcholine release at synapses and neuromuscular junctions. The toxin's lethal dose is 0.01 grams making this the most potent toxin known to man. Symptoms occur 3-96 hours post ingestion (18 hours is the norm). Symptoms include headache and weakness, a progressive paralysis downward from eyes to face to neck to thorax, double vision and photophobia.

The pulse rate slows and there is a drop in body temperature. Eventually death occurs because of respiratory paralysis. In adult botulism (food intoxication) the POE is ingestion of toxins especially in canned foods. In this anaerobic environment, spores germinate when the cans are improperly sterilized. Low acid (pH < 4.5) foods are especially dangerous but can occur in meats that are in plastic wrappers. Prevention of toxin production can be achieved by high salt (8%), sugar (50%) in absence of proper processing.

Infant botulism (food infection), the most common form in U.S. today is a result of ingestion (often in honey) of the organism. The organism multiplies in the infant's immature gut and produces toxin that is absorbed into the bloodstream. Wound botulism occurs when the POE is deep, soil-contaminated wounds. Symptoms are the same. Treatment must start before symptoms appear and uses antitoxin to bind the toxin. A toxoid is available and used for animals as a vaccine. The need for boosters and nature of infection makes the vaccine impractical for humans. Inhalation of botulinum toxin has become a concern in this age of bioterrorism.

Clostridium difficile is a gram-positive bacillus, with peritrichous flagella and an oval, subterminal spore. The organism is an obligate anaerobe that causes diarrhea and antibiotic-associated pseudomembranous colitis. Toxin A (enterotoxin) causes diarrhea and toxin B (cytotoxin) attacks the mucosa of colon to form pseudomembrane. Together they cause dehydration, septicemic shock, death. The organism is normal flora in infants and in 5% of adults. It is resistant to most antibiotics. Nosocomial infections are involved in most cases. Spores are easily transmitted. Antibiotic use causes opportunistic infections in the elderly. Vancomycin and metronidazole are used in therapy.

The rest of the gram positive bacilli do not form spores.



Listeria monocytogenes is gram-positive single coccobacilli to palisade forms in chains. They possess one to four flagella, causing a characteristic tumbling motility. This organism is a facultative anaerobe that survives in cold temperatures, easily cultured and weakly beta hemolytic on blood agar where it resembles Group B Strep both morphologically and the disease it produces in the newborns. The primary infection of listeriosis is in the GI tract where the organism is acquired through ingestion of contaminated dairy and meat products. The organism thrives in refrigeration. It generally causes a mild fever, sore throat and diarrhea. However, secondary infections can occur in debilitated patients when the infection extends to brain and meninges and bloodstream (septicemia with high fatality). If the patient is a pregnant female, transplacental infection can cause a spontaneous abortion. Treatment requires antibiotic therapy with ampicillin, erythromycin or sulfa drugs. Vaccines are not available.

Erysipelothrix rhusiopathiae is an alpha hemolytic, gram positive bacillus. It causes erysipeloid in humans and erysipelas in swine. Therefore humans at greatest risk are those who handle animals, carcasses, and meats. This is a zoonosis.

Corynebacterium diphtheriae is a gram-positive pleomorphic bacillus. This nonmotile, non spore-forming bacillus is a facultative anaerobe that grows best on blood agar. However, selective media are required to isolate the organism. It causes a disease called diphtheria. The primary infection is nasopharyngeal but 75% of the population has the organism as normal flora. Pathogenic strains are activated by transduction via a bacteriophage which carries a gene for the exotoxin production. The disease is due to the exotoxin (Toxin fragment A prevents protein production in cells alone). A pseudomembrane forms in pharynx. Dead cells accumulate in fibrinous

exudates and enlargement of the membrane may block larynx and trachea. Eventually a toxemia ensues that is absorbed by mucosa. The Toxin fragment B attacks the peripheral nervous system and/or heart. Death results from heart involvement (cardiac arrhythmia) or respiratory paralysis. Man is the only known host. The POE is via the respiratory system from inhalation of droplet sprays. Organisms can live 6 months in dried sputum. Treatment involves penicillin and antiserum. Prevention via a toxoid vaccine (part of DPT) and boosters offer protection.

Mycobacterium tuberculosis (MTB) is an ACID-FAST organism that stains irregularly with the Gram Stain. Acid-fast organisms retain the stain in the presence of a weak acid. Its cell wall is rich in lipids. It forms branching chains of rods that are nonmotile. The organism is a strict aerobe that requires a selective media because of extremely slow growth. MTB causes the disease tuberculosis, a lifelong infection, which primarily is an infection at the base of the lung. One-third of the world has a MTB infection and over two million people die each year from tuberculosis disease. One in ten of them will become sick with active TB in their lifetime.

The organism enters the lungs, yet the cellular arm of the immune system cannot kill the organism. The growth of tubercle (nodules of clear material surrounding the organism) indicates phagocytic activity by macrophages causing a caseation. Fibroblasts wall off tubercle. Cavitation causes drainage of the tubercle that is resolved by fibrosis and calcification. If the immune system weakens, as it often does with age, poor nutrition and other diseases, secondary infections occur, transmitted via the bloodstream and involve any organ in body. TB has been called a wasting disease because of the weight loss patients have during the disease process. The outcome depends on organ involved, but can be fatal. Transmission of primary infection is via respiratory droplets or contaminated milk from cows. Treatment requires use of synergistic drugs such as INH, EMB, Rifampin, and Streptomycin. A vaccine BCG, (attenuated bovine strain) is available but vaccination prevents diagnosis using skin tests (T-cell). Vaccination thus causes a false positive reaction but does protect against severe childhood forms of the disease. A major concern today is the global outbreak of multidrug-resistant tuberculosis.

Mycobacterium leprae is another ACID-FAST organism that produces branching chains of bacilli. Even though this is an obligate intracellular parasite, it cannot grow on artificial media. The foot pads of mice or an armadillo are used to culture this organism. *M. leprae* causes leprosy (Hansen's disease). The primary infection is a result of either cutaneous or respiratory POE after a long incubation period. Nerves are involved almost immediately to create symptoms. In the tuberculoid variety, rapid

colonization of Schwann cells causes significant anesthesia and disfigurement. Normal macrophage and T-cell response helps limit disease progression. In the lepromatous variety, organisms multiply in macrophages amid high level of suppressor T-cells. There is slow nerve involvement with painless atrophy of muscle, skin and bones. This form has a high fatality rate due to abnormal immune response of other T-cells. Secondary infections are the usual cause of fatalities because the abnormal immune system cannot fight them off. The lepromatous form is more infectious than the tuberculoid form.

Isolation of patient was once the main method of prevention. Carville, Louisiana and Molokai, Hawaii still have leper colonies. The World Health Organization estimates 750,000 people are being treated for Hansen's disease worldwide, mostly in India, Burma, Nepal, Africa and South America. Once treatment with sulfone and rifampin synergistically begins, organisms are not shed. BCG vaccine has been used with some success.

Mycobacterium avium-intracellularae complex (MAC) frequently cause secondary infections in AIDS patients and require multiple antibiotics to treat.