

## **Class 1 & 2: Introduction, Anthrax as a model of Disease**

### **Anthrax and It's Cycle**

An infectious disease of ruminants resulting from the ingestion of spores of *Bacillus anthracis*. Spores are persistent in soil for decades. Pasteur invented the first vaccine by growing *B. anthracis* at elevated temperatures.

- ***B. anthracis***: Gram-positive, aerobic or facultative anaerobic, endospore forming bacteria. Rod-shaped and approximately 4µm by 1µm. Frequently appears in chains.

The bacteria normally rests in spore form in the soil, and can survive for decades in this states. Once ingested by a ruminant, the bacteria begins multiplying inside the animal and eventually kills it, then continues to reproduce in the carcass. Once the bacteria consume the host nutrients, they revert to a dormant spore state. Cows are vaccinated as an attempt to control anthrax outbreak.

### **Cutaneous Anthrax**

Begins with an initial papule with an oedematous change around day 3 and a formation of vesicle ring around the papule with clear fluid. In the later stages, an eschar (thick scar) forms, which blackens and eventually grows over the vesicles and thickens.

### **Pulmonary Anthrax**

Otherwise known as Woolsorters' Disease because anthrax inhalation is commonly experienced by workers exposed to Anthrax spores from animal hides and furs. Occurs with high inoculum, and abrupt onset and progression, death occurs within 2-3 days. Antibiotics are only useful immediately after exposure. Lesions appear in (mediastinal) lymph nodes draining the lungs.

### **Diagnosis**

- Demonstration of *B. anthracis* in a clinical specimen by microscopic examination of stained smears of vesicular fluid, pleural fluid, stools etc.
- Isolation of *B. anthracis* from a clinical specimen (eg. blood, lesions, discharges).
- Positive serology (ELISA, Western blot, toxin detection, chromatographic assay, fluorescent antibody test (FAT)).

### **Incidence of Anthrax**

In view of associations between times of higher incidence and dry, hot conditions, theories have arisen that at such times, the animal is forced to graze dry, spiky grass close to the soil. The spiky grass and grit produce gastrointestinal lesions and if the soil is contaminated with anthrax spores, there is a high chance of infection occurring.

### **Anthrax and Bioterrorism**

*B. anthracis* is one of the most important pathogens on the list of bioterrorism threats. The human LD50 for inhalation exposure is about 8000 to 40000 spores or one deep breath at the site of release. In 1993 the bacteria was aerosolised from the roof of an 8 story building in Japan. In the US in 2001, letters containing anthrax were mailed to several news media offices and two US senators, killing 5 people and sickening 17 others. After this letters to US government are now irradiated to kill anthrax spores, although long term effects of this radiation are unknown.

## Pathogenesis

The spore is located and engulfed by phagocytes (macrophages and dendritic cells). Inside the phagocytic cell, the spore turns into a bacillus, multiplies and eventually bursts the cell, releasing bacilli into the bloodstream.

Vegetative cells are released into the extracellular milieu and enter the circulation where the vegetative cells grow to levels as high as  $10^8$  bacteria per ml of blood. In this environment the vegetative bacteria respond to physiological body temperature and  $\text{CO}_2$  levels by activating genes responsible for capsule formation and toxin synthesis.

Finally, massive edema and organ failure are produced as a consequence of toxin formation.

The virulence of a strain of anthrax is dependent on multiple factors, primarily the poly-D-glutamic acid capsule that protects the bacterium from phagocytosis by host neutrophils and its toxins, oedema toxin and lethal toxin.

Tripartite toxin consists of three polypeptides: Protective antigen, lethal factor and oedema factor. PA binds ubiquitously to Tumour Endothelium Marker-8 (TEM8) that then internalises the toxin. The two components (OF and LF) of the toxin enzymatically modify substrates within the cytosol of the mammalian cells. OF is an adenylate cyclase, the LF is a zinc dependent protease that cleaves several mitogen activated protein kinase kinases (MAPKK) and causes lysis of macrophages.

## Anthrax Toxin

Three components: EF, LF, PA. Recent evidence indicates that anthrax also targets endothelial cells, causing vascular leakage (similar to haemorrhagic bleeding), and ultimately hypovolemic shock, not septic shock.

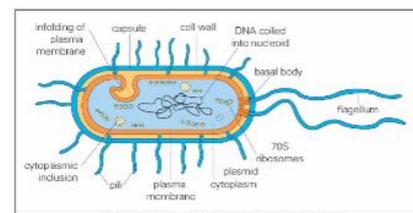
Although the bacteria can be treated by antibiotics, this chemotherapy frequently fails in particular if not initiated immediately after infection, because even after eradication of the pathogen, bacterial toxins can remain in circulation. Inhaling anthrax has a fatality rate of 45-90 percent.

## Class 3: The Bacteria

- **Parasite:** An organism that lives in or on the living tissue of a host organism at the expense of that host.
- **Pathogen:** A specific biological causative agent of disease.

Bacteria are single cell organisms that exist either as free living cells or colonies. Bacteria have both a cell membrane and a cell wall that protects them from their environment. They all share common biochemical pathways (eg glycolysis) with eukaryotic cells, but also have unique pathways that are targets for antibiotics.

## Generalised Bacterium



The bacterial cell wall comes in two forms that are distinguished by the 'gram stain'. Gram positive bacteria have a simpler wall structure with a relatively large amount of peptidoglycan. Gram negative bacteria have a more complex cell wall structure. The gram stain uses two dyes: One purple, the other red. The purple dye binds strongly to the cell wall of Gram positive bacteria, but is easily washed out of the wall of Gram negative bacteria, leaving these a red colour.