

A Sin of Biotechnology, Bioterrorism - Anthrax

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Abstract: Anthrax is an acute disease of both humans and animals. It is caused by a rod shaped, spore forming gram-positive bacteria, *Bacillus anthracis*. The spores play a vital role in the transmission of this fatal disease "Anthrax". The aerosols of spores of *Bacillus anthracis* is a chosen weapon of bioterrorism. The bacillus affects the parts of human body like the intestine, skin and lungs causing Gastrointestinal, Cutaneous and Inhalation anthrax respectively. Large doses of intravenous and oral antibiotics such as flouoroquinolones like Ciprofloxacin, Doxycycline, Erythromycin, Vancomycin and Penicillin are recommended for the treatment of anthrax in the early stages, else the condition turning fatal. Various vaccines formulated to prevent the spreading of fatal disease anthrax are Biothrax, Strene strain, preparation of protective antigen and Passive vaccine.

Key words: Anthrax, *Bacillus anthracis*, Antibiotics, Biothrax, Bioterrorism.

Introduction

Anthrax comes from Greek word anthracite (coal) in reference to the black skin lesions, the victims develop in the cutaneous skin infection. It is an acute disease of humans and animals caused by rod shaped gram-positive spore forming bacteria, *Bacillus anthracis*. The bacterium rests in endospores form in the soil, water, dust and air. It is infected to the animals when the spores are ingested or inhaled. In humans, it is infected when they were exposed to blood and other tissues from infected animals¹.

Anthrax affects parts of human body like intestine, lungs and skin causing gastrointestinal, inhalational and cutaneous anthrax respectively. In the United States the incidence of naturally acquired anthrax is extremely rare i.e, 1 to 2 cases of cutaneous disease per year. In the year 2001 intentional contamination lead to 22 cases of anthrax (11 cutaneous, 11 inhalational) in United States. Therefore, the spores of *Bacillus anthracis* are found to be a choosen weapon of bioterrorism².

Morphology of *Bacillus anthracis*

Bacillus anthracis is a rod shaped gram-positive bacteria. It is the first pathogenic bacterium to be observed under the microscope, first bacillus to be isolated in culture. It is gram-positive and non acid-fast bacterium³, measuring 3-10 µm and 1-1.6 µm. In tissues, it is found singly, or in pairs or in short chains and the entire chain is surrounded by a capsule. The capsule in a polypeptide composed of d (-) glutamic acid. The bacterium can be cultivated in ordinary nutrient medium containing bicarbonate, under aerobic or anaerobic conditions or incubated under 10-25% carbon dioxide. The chain of bacilli presents a "bamboo stick" appearance. Sporulation is inhibited by calcium chloride.

Spores are central or sub-terminal, elliptical or oval in shape and are of the same width as the bacillary body. The spores are stained with sudan black B, fat globules are formed within the bacilli. When the spores are once ingested or placed in an open cut, the bacterium begins multiplying inside the human or

animal and kills the host within a few days or weeks. The endospores germinate at the site of entry into the tissues and spread via the circulation to the lymphatics. It produces the two powerful exotoxins⁴ namely edema toxin and lethal toxin which causes death.

Types of Infection

Gastro intestinal Anthrax

Gastrointestinal anthrax is caused by eating the cooked meat from infected animals. Intestinal anthrax has an extremely high mortality rate. It is characterized by serious gastrointestinal difficulty, like vomiting of blood, severe diarrhoea, acute inflammation in the GI tract and loss of appetite⁵. Some lesions are found in the mouth and throat, the organism invades through a pre-existing lesion and spreads to the lymphatic system throughout the body, making more toxins on the way.

Cutaneous anthrax

It is also called as “Hide Porter’s disease”. It is infected through the skin and characterized as a boil like skin lesion which forms an ulcer with black eschar. It shows as a large, painless necrotic ulcer at the site of infection. It forms within the site of spore penetration within 2-5 days after exposure. It is rarely fatal and cause to toxemia and death⁶.

Pulmonary anthrax

It is called as “Wool sorter’s disease” because it is used to be common in workers in wool factories, due to the inhalation of dust from infected wool, handling of hair bristles used for the manufacturing of brushes etc^{7, 8}. Optimum size of spore is about 1-5micrometer in diameter. The inhaled spores are carried to lymph nodes by phagocytes, where the spores germinate and toxins are released which cause hemorrhagic mediastinitis or hemorrhagic meningitis. Inhalation anthrax rapidly leads to shock and frequently death within 1-2 days.

Pathogenicity

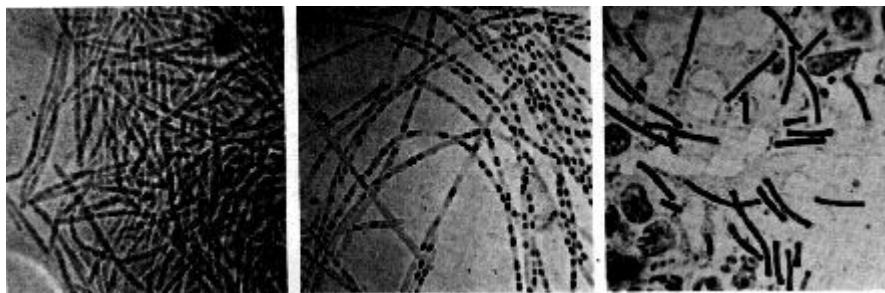
Anthrax is a disease of both animals and humans, with two major determinants of virulence having been identified. The formation of a poly-D-glutamyl capsule, which mediates the invasive stage of the infection and the production of the multi component anthrax toxin, mediates the toxinogenic stage⁹.

Bacillus anthracis forms a single antigenic type of capsule consisting of a poly-D-glutamate capsule. Production of capsular material is associated with the formation of a characteristic mucoid colony type. Capsule production depends on a 60 mega dalton plasmid, px02; its transfer to non-encapsulated, via transduction produces the encapsulated phenotype¹⁰. The capsule plays its most important role during the establishment of the infection and a less significant role in the terminal phases of the disease, which are mediated by anthrax toxin.

Production of anthrax toxin is mediated by a temperature-sensitive plasmid, px01, of 110 mega daltons^{11, 12}. The toxins consists of three distinct antigenic components namely edema factor (EF), protective antigen (PA) and lethal factor (LF). They are not toxic individually but the combinations of these produce local edema and generalized shock¹³.

Diagnosis

Anthrax may be diagnosed by microscopy culture, animal inoculation and serological demonstration of the anthrax antigen in infected tissues. The specimen used for diagnosis is swabs, fluid or pus from pustules, sputum and blood. In the microscopic determination, the spores are stained with Sudan black B, fat globules is formed within the bacilli. The capsular material can be detected by the McFadyean reaction which involves staining with polychrome methylene blue¹⁴. A blue rod in a background of purple pink stained capsular material is a positive test.



Robert Koch’s original photographs of *Bacillus anthracis*, the agent of anthrax.

In the culture diagnosis the smears shows the large gram –positive bacilli in the nutrient agar for the appearance of medusa head colonies. Gelatin stab culture shows the inverted tree growth. In the animal inoculation technique, when an animal is suspected to have died of anthrax, autopsy is not permissible, as the split blood will lead to contamination of the soil. An ear may be cut off from the carcass and sent to the laboratory. Alternatively, swabs soaked in blood or several blood smears may be sent. In Serological tests, Antibiotics to organism can be demonstrated by gel diffusion, complement fixation and ELISA technique,¹⁵ Ascoli's thermo precipitin test is used for the rapid demonstration of anthrax antigen in tissue extracts which shows the ring of precipitate at the junction of two fluids within five minutes in a positive case.

Prevention

Vaccines composed of killed bacilli and capsular antigens produce no significance immunity. The Sterne strain of *Bacillus anthracis* produces sub lethal amounts of the toxin that induces formation of protective antibody.

The original Pasteur's anthrax vaccine is of great historical importance. Pasteur's vaccine was the anthrax bacillus attenuated by growth at 42-43°C. As the spore is the common infective form in nature, vaccines consisting of spores of attenuated strains were developed. The vaccine consists of spores of stable attenuated Carbazoo strain in 2% saponin.

The vaccine for humans is a preparation of protective antigen (PA) recovered from the culture filtrate of a virulent, non encapsulated strain of *B. anthracis* that produces PA during active growth. Active immunization consists of 3 sub-cutaneous injections given two weeks apart followed by three additional sub-cutaneous injections given at 6, 12 and 18 months. Annual Booster injections of the vaccine are required to maintain a protective level of the immunity¹⁶. A new type of passive vaccine to anthrax is currently on the horizon. This forms antibodies directly to the body and immunity is achieved much sooner. Alum precipitated toxoid prepared from protective antigen has been shown to be a safe and effective for human use^{17, 18}.

Component of tea, such as poly phenols have the ability to inhibit the activity of both *B.anthraxis*

and its toxin considerably. However, addition of milk to the tea completely inhibits its antibacterial activity against anthrax¹⁹. Aerial spores can be trapped by a sample HEPA or P100 filter and thereby, inhalation of anthrax spores can be prevented²⁰.

Treatment

For the treatment of anthrax, antibiotics should be given to unvaccinated individuals exposed to anthrax. Large doses of intravenous and oral antibiotics such as flouroquinolones like Ciprofloxacin, Doxycycline, Erythromycin, Vancomycin or Penicillins are recommended. Once the toxin is formed the antibiotics becomes ineffective²¹.

Dose: Doxycycline 100 mg is recommended for every 12 hrs. Incase of inhalational and gastrointestinal anthrax, doxycycline and ciprofloxacin must be used in combination.

Biological warfare

The inhalational of an anthrax spores can lead to infection and disease. The aerosols containing anthrax spores are chosen as weapon of bioterrorism. As an agent of biological warfare it is expected that a cloud of anthrax spores would be released at the strategic location to be inhaled by the individuals under attack.

In 2001 anthrax attacks in the United States, delivered mailing postal letters containing the spores. Only a few grams of material were used in these attacks²². In response to the postal anthrax attacks and hoaxes, the U.S postal services were sterilized. Some mail using a process of gamma irradiation and treatment with a proprietary enzyme formula supplied by Sipco industries Ltd.²³ The domestic electric iron at its hottest setting at least 204°C used for at least 5 minutes should destroy all anthrax spores in a common postal envelope.²⁴

Discussion and Conclusion

Though anthrax is preventable in these days due to the development of various preparations like strene strain of *B. anthracis*, preparation of protective antigen, passive vaccine etc. Further investigation and development has to occur in these departments of vaccination to produce vaccination for the eradication of this dreadful disease.

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