

Anthrax – Clinical characteristics and use as a biological warfare agent ¹

by
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AETIOLOGY

ANTHRAX IS CAUSED BY the microorganism *Bacillus anthracis*, a large Gram-positive rod-shaped bacterium which is commonly found singly or in pairs. The organism is capsulated in clinical specimens, but endospores are produced in vitro, in soil, and in decaying animal tissue.

These endospores are relatively resistant to heat and chemical disinfectants (they can be destroyed by boiling for 30 minutes or more, or exposing to 140°C dry heat for three hours), but may remain viable for months in animal hides or for years or decades in dry earth. ¹

EPIDEMIOLOGY

B. anthracis is found worldwide, particularly in Asian and African countries. It is widespread in south-eastern Australia due to distribution by dust storms and wild pigs and dogs.

Anthrax is naturally an infectious disease in farm animals occasionally transmitted to man, usually by inhalation or ingestion of spores or via sub-cutaneous abrasions. Almost all animals are susceptible, especially herbivores. There is no true reservoir, but spores may remain viable in soil.

Man-to-man transmission is extremely unlikely.

PATHOLOGY

Three principal antigens are associated with the pathogen.

Capsular antigen

D-glutamic acid polypeptide formed by virulent strains of *B. anthracis* in infected tissue. The capsule is anti-phagocytic and protects the bacterium from lytic antibodies. It is important in pathogenicity and in the establishment of infection. The gene coding for this antigen resides on a plasmid known as pX02. ²

Somatic (Cellular) Antigen. Polysaccharide of equal proportions in D-galactose and N-acetylglucosamine in the cell wall.

Anthrax Toxin.

Complex toxin produced in vivo mediated by a temperature-sensitive plasmid (pX01) ³ consists of a protective antigen (PA) (Mwt 85,000), lethal factor (LF) (Mwt 83,000), and oedema factor (OF) (Mwt 89,000) ⁵ - a combination of these factors produces toxicity. The toxin is responsible for the symptoms of the disease.

PA is the most important toxin in protection and contains the major immunogenic epitopes. It binds to the cell surface, where it undergoes proteolytic cleavage, exposing a site to which OF and LF bind. The complex is then internalised, probably by endocytosis. ⁶ It is believed that the oedema factor causes an increase in the amount of cyclic AMP in the cytoplasm of the host cell ⁷.

Virulence is dependent of the production of toxin and the presence of a capsule. Accumulation of toxin in tissues affects the central nervous system, which may result in respiratory failure and anoxia.

Antibiotic therapy may sterilise tissue, but toxin may persist until is metabolised, prolonging the clinical disease.⁸

CLINICAL MANIFESTATIONS

Three different presentations of anthrax occur, depending on the route of infection. All can progress to fatal bacteraemia by dissemination via the blood- stream. Meningitis sometimes occurs as a complication of severe cases.⁸

Cutaneous Anthrax.

This is the most common form of the disease in humans. Spores penetrate the skin via minor cuts or abrasions, which may become itchy. When the spores germinate -two to five days after exposure - an inflamed papule appears at the site of inoculation. Pus is usually not present unless a secondary infection is involved.

Within a few days, a vesicle (called a malignant pustule) forms, filled with a bluish-black fluid. This vesicle will eventually break down, being replaced with a black eschar with gelatinous surrounding oedema (this lesion is not usually painful). The eschar will dry out after one to three weeks, separating from the surrounding skin and leaving a scar.^{8,9}

If untreated, or in extremely severe cases, cells, may spread to regional lymph nodes, which may become enlarged and tender, and invasion of the bloodstream by the pathogen may follow.'

Mortality in untreated cases is between five and twenty per cent, and under-five per cent if antibiotic therapy is prompt.

Inhalation Anthrax.

One to five days after inhalation of spores, common respiratory symptoms develop (fever, non-productive cough, myalgia, malaise). Spores are phagocytosed by macrophages and carried to regional tracheobronchial lymph nodes, where they germinate and rapidly multiply.¹⁰

Although an apparent improvement may occur, symptoms abruptly worsen after a few days; high fever, dyspnoea, cyanosis, chest and neck oedema, respiratory stridor, chest pain and pleural effusion are common. Haemorrhagic oedema tousmediastinitis often occurs and may develop into haemorrhagic meningitis.^{5,8} Anthrax toxin may directly affect the pulmonary capillary endothelium which may result in thrombosis and respiratory failure.

A few bacteria are usually able to evade the host's cellular defences and escape into the bloodstream via the efferent lymphatic. They are cleared by the reticuloendothelial system (especially the spleen) but are able to establish a fatal bacteraemia.⁵

The patient's condition rapidly deteriorates, leading to respiratory distress, cyanosis, and death usually within 24 hours.^{5,8} Unless the disease is identified and antibiotic therapy started within 12 hours after inoculation, inhalation anthrax is usually always fatal.

Chest x-ray shows distinct mediastinal widening.¹² Pneumonia caused directly from anthrax does not occur, but secondary infection causing pneumonia may result.^{5,11}

Gastrointestinal Anthrax.

This is an extremely rare disease, following ingestion of spores in contaminated, undercooked meat. Deposition and germination of spores in the submucosa of the ileum and caecum and subsequent toxin production may cause

oedema, haemorrhage and necrosis, and result in nausea, vomiting and diarrhoea. The incubation period is usually between two and five days.⁸

In severe cases, cholera-like gastroenteritis may follow, with abdominal pain, fever, bloody vomitus and diarrhoea, intestinal obstruction, prostration and shock. Haemorrhagic inflammation of the small intestine and bowel perforation may also occur. These symptoms are associated with a very high mortality rate (25% to 75%).⁵

Regional lymph nodes may become infected, leading to a systemic infection.

Very rarely, tonsillar or pharyngeal ulceration may also be evident. Formation of a pseudomembrane, followed by difficulty in swallowing and respiratory compromise may result.'

DIAGNOSIS

Laboratory Diagnosis.

Gram stains and immunofluorescent antibody assays on pustule exudates or blood are useful. Sputum is generally not suitable as spores do not usually germinate until they reach the lymph nodes.

Blood samples should be cultured, although specimens from cutaneous tissue, lymph nodes, sputum or CSF may also be suitable. *B. anthracis* grows on routine media, especially blood agar, and has characteristic grey-white, irregular, hair-like colony forms when grown optimally in aerobic conditions at 37" C.^{5,13}

Serology on paired sera is only worthwhile as confirmation.

Differential diagnosis.

CUTANEOUS ANTHRAX: orf, plague, tularaemia, staphylococcal carbuncle.

INHALATION ANTHRAX: initially influenza or any of a wide range of bacterial, viral or fungal URI infections. Very hard to recognise promptly, although a BW attack would probably result in an explosive outbreak⁹.

Look for mediastinal widening in chest x-ray, chest wall oedema, haemorrhagic pleural effusions and haemorrhagic meningitis.

May be confused with an aerosol attack of Staphylococcal B enterotoxin (SEB), although the onset of symptoms of SEB would be more rapid, and no mediastinal widening would be present.¹¹

Plague pneumonia also has similar symptoms to inhalation anthrax, but these patients would have pulmonary infiltrates, which are usually absent in anthrax.

GASTROINTESTINAL ANTHRAX: acute abdomen, appendicitis, gastroenteritis.

TREATMENT

The antibiotic of choice is penicillin-G administered parenterally, although tetracycline is also effective. Most strains are also sensitive to erythromycin and chloramphenicol.^{5 8 11}

Cutaneous lesions should not be excised and drained, as this may lead to dissemination of the pathogen into the bloodstream and septicaemia. The patient should be isolated, and the lesion kept sterile and dry. Skin grafts may be necessary after the infection has resolved. Corticosteroids are sometimes used to treat severe malignant pustules.

Pulmonary anthrax is usually diagnosed too late for antibiotic therapy, although administering both antibiotics and antitoxin may be of some benefit. Therapy should be continued for a prolonged period of time.⁸

RECOMMENDED THERAPY

2 x 10⁶ units of penicillin-G every 2 to 6 hours until oedema subsides, followed by oral penicillin for at least 7 to 10 days. Erythromycin, tetracycline, or chloramphenicol may be used in penicillin-sensitive patients.

In the event of a BW attack where multiple drug-resistant strains of *B. anthracis* have been used, treatment should be given as follows:

1,000 mg ciprofloxacin orally at the first sign of the disease, followed by 750mg orally twice daily
OR
200 mg intravenous doxycycline initially, then 100 mg twice daily.

Unvaccinated personnel should also be given a single 0.5 ml dose of vaccine subcutaneously. Two additional doses of 0.5 ml should be given two weeks apart.

Personnel vaccinated with fewer than three doses should receive a single 0.5 ml booster.¹¹

Antibiotic therapy should be continued for at least four weeks. If the vaccine is not available, antibiotics should be continued for a prolonged period of time.

If possible, the surrounding environment should be decontaminated: formaldehyde is effective for sterilizing soil and equipment. (Gamma radiation has proven to be effective in factory decontamination, but ethylene dioxide and autoclaving do not appear to be as efficient)⁸

SUSCEPTIBILITY OF POPULATION

Susceptibility is very high in unvaccinated individuals. There appears to be no documented evidence for differences in susceptibility between males and females.

PREVENTION

Several different vaccines are suitable for human use; either live attenuated or killed vaccines. All efficient vaccines either contain or produce PA; neither LF nor OF are protective by themselves.

Non-living PA Vaccines.

Effective vaccines of protective antigen adsorbed onto an aluminium hydroxide adjuvant (from the US)¹⁴ or alum precipitated (from the UK)¹⁵ are available and appear to give protection against inhalation anthrax (although protection against large challenges or highly virulent strains of *B. anthracis* may not be afforded).

Doses at 0, 2 and 4 weeks, 6, 12 and 18 months and then every year are recommended. Protection seems to be acquired after the third dose (limited data available). These vaccines are the best option at present. Antibodies are produced in virtually all patients after the 12-month booster.

Reaction to the vaccine is usually only mild to moderate, with tenderness, erythema, oedema and pruritus. More severe complications are rare (less than 1 % of cases) but may limit the patient's use of the extremities for 1 to 2 days, and induce myalgia, malaise, or low-grade fever. Severe systemic reactions (anaphylaxis) are very rare.

The vaccine should be stored at 4°C - NOT frozen. NB: No definitive field trials have been performed to evaluate vaccine efficiency, and no information is available correlating specific immune response to vaccination and protection afforded, particularly with respect to aerosol challenge, or against virulent strains of *B. anthracis*.

[Editor's Note: There have been subsequent research papers in Vaccine and a review by the Institute of Medicine in this area]

Live Attenuated Spore Vaccines.

A live spore vaccine (STI- derived from the Sterne spore vaccine) from the former USSR of a non-encapsulated attenuated strain which lacks the pX02 plasmid is also available. This vaccine is administered by scarification or even by aerosol¹⁶ Boosters are needed every year. Although protection is likely to be higher than that of the killed vaccine, no conclusive comparative experiments have been conducted.¹⁷ Severe reactions are common- necrosis at the site of inoculation sometimes occurs.

If the patient survives the disease, recovery from anthrax provides solid immunity.

Recently, recombinant vaccines, and more efficient delivery systems of the PA antigen are being developed (see Future Directions).

POTENTIAL AS BW

Advantages as a BW.

An aerosol attack of B. anthracis would cause inhalation anthrax, which has a short incubation period, is difficult to recognise promptly and, unless diagnosed early, would produce fatalities.

B. anthracis is easy to cultivate in large numbers in the laboratory, which would enable third world countries to acquire a large stock.

The protective qualities of the endospore allow stability of the pathogen in sunlight (for several days), in soil (for decades), and in aerosol form.

The hardiness of the bacteria is its ability to survive in harsh environments, and the ease with which it can be distributed via the wind would be attractive if widespread dissemination of the pathogen is desired and long-term contamination of the environment is not deemed important (e.g. in terrorist activities)⁸

A small scale attack may be able to target key personnel, but without extensive contamination of the environment, and disinfection of the area would be possible.⁸

Antibiotic-resistant strains are easily engineered in the laboratory.

Current vaccines may not provide enough protection against a large challenge of spores, and the pro-longed therapy necessary following an attack could seriously deplete antibiotic stocks and medical resources.

Disadvantages as BW

Spores persist for prolonged periods of time, and their dissemination is difficult to control, which would be detrimental if the target area was of importance.

Protection of own personnel would be necessary.⁸

FUTURE DIRECTIONS

Vaccines

Several different approaches are being researched. Ideally, vaccines should:

- be safe to humans;
- broadly protective after only one dose; effective, with negligible side effects;
- and
- give rapid and long-lasting immunity.

Novel approaches involve both non-living and living vaccines.

Non-living Vaccines

Non-cellular vaccines containing PA which give strong, protective immune responses are being trialled. Several aspects of these vaccines must be examined.

EPTTOPE ANALYSIS. The structure and function relationships of the anthrax toxins (most notably PA), as well as the molecular interactions between the toxins, are currently being elucidated. If those epitopes which produce strong immune responses can be determined, synthetic peptide vaccines may be feasible. Ideally, these vaccines would effectively stimulate cell-mediated immunity, but with negligible side-effects.

DEVELOPMENT OF SUITABLE ADJUVANTS. Many adjuvants are successful as immune stimulants in animal vaccines but are unsuitable for human use because of unacceptable side-effects. Current adsorbed PA vaccine has increased efficacy when used with complete Freund's adjuvant, Corynebacteriumovis, or killed Bordetella pertussis.¹⁸ However, these adjuvants are associated with allergic reactions in humans.

Recently, potential biological adjuvants have been investigated, which give a high stimulatory effect, but are otherwise innocuous. A bacterial peptidoglycan moiety (N-acetyl muramyl-L-alanine- D-isoglutamine (MDP)) has been shown to be effective¹⁹ and synthetic muramyl dipeptide derivatives have been made.²⁰ Other compounds, such as dimethylglycine (DMG), threonyl MDP, monophos- phoryllipid A, trehalose dimycolate, and cell wall components of Mycobacterium phlei and M. Bovis may also have potential.^{21,22}

VIRUS-EXPRESSED PA. Two different approaches are being investigated, using either baculovirus or vaccinia virus expression systems.²³ **BACULOVIRUS SYSTEM.** The PA gene is cloned into the baculovirus genome and the virus is used to infect insect cell tissue culture. PA is produced and purified and injected into guinea pigs and mice.

VACCINIA VIRUS SYSTEM. The experimental animals are immunised with the PA-vaccinia recombinant which replicates and produces PA.

Both methods induced a high degree of protection in the animals against a highly virulent (Ames) strain of B. anthracis.

The PA produced was immunologically identical to the Sterne spore PA.

GENETICALLY ENGINEERED VACCINES. Site-directed mutagenesis of toxin genes may produce modified toxins (PA, OF, LF, and somatic antigens) which have enhanced immunogenicity but a decreased toxicity. These could be used as either living or non-living vaccines.²¹

Live Attenuated Vaccines

Several mutant B. anthracis strains have been examined as possible live vaccines. Transposon (Tn) mutagenesis, which has proven successful with Salmonella typhimurium vaccines, is now being applied to B. anthracis.

B. anthracis Tn916 mutants (aro mutants) of a non-encapsulated, toxigenic strain are unable to synthesise the aromatic amino acids phenylalanine, tyrosine and tryptophan, and only replicate a few times in the host. This self-limiting infection is enough to afford protection in guinea pigs against virulent strains of the pathogen.²¹

OTHER TRENDS

Research into antibiotic prophylaxis, particularly with regard to better delivery systems (such as liposome-encapsulated drugs for improved persistence in the host) is occurring. Passive immune approaches are not yet feasible for human use.

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