Chapter 22

ANTHRAX

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SUMMARY

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INTRODUCTION AND HISTORY

Anthrax, a zoonotic disease caused by *Bacillus anthracis*, occurs in domesticated and wild animals—primarily herbivores, including goats, sheep, cattle, horses, and swine. Humans usually become infected by contact with infected animals or contaminated animal products. Infection occurs most commonly via the cutaneous route and only very rarely via the respiratory or gastrointestinal routes.

Anthrax has a long association with human history. The fifth and sixth plagues described in Exodus may have been anthrax in domesticated animals followed by cutaneous anthrax in humans. The disease that Virgil described in his *Georgics* is clearly anthrax in domestic and wild animals. And during the 16th to the 18th centuries in Europe, anthrax was an economically important agricultural disease.

Anthrax was intimately associated with the origins of microbiology and immunology, being the first disease for which a microbial origin was definitively established, in 1876, by Robert Koch. It also was the first disease for which an effective live bacterial vaccine was developed, in 1881, by Louis Pasteur. During the latter half of the 19th century, a previously unrecognized form of anthrax appeared for the first time, namely, inhalational anthrax. This occurred among woolsorters in England, due to the generation of infectious aerosols of anthrax spores under industrial conditions, from the processing of contaminated goat hair and alpaca wool. It probably represents the first described occupational respiratory infectious disease.

Owing to the infectiousness of anthrax spores by the respiratory route and the high mortality of inhalational anthrax, the military’s concern with anthrax is with its potential use as a biological weapon. This concern was heightened by the revelation that the largest epidemic of inhalational anthrax in this century, in Sverdlovsk, Russia, in 1979, occurred after anthrax spores were released from a military research facility located upwind from where the cases occurred. Cases were also reported in animals located more than 50 km from the site.

THE ORGANISM

*Bacillus anthracis* is a large, Gram-positive, spore-forming, nonmotile bacillus (1–1.5 µm x 3–10 µm). The organism grows readily on sheep blood agar aerobically and is nonhemolytic under these conditions. The colonies are large, rough, and grayish-white, with irregular, curving outgrowths from the margin. Both in vitro in the presence of bicarbonate and carbon dioxide, and in tissue in vivo, the organism forms a prominent capsule. In tissue, the encapsulated bacteria occur singly or in chains of two or three bacilli (Figure 22-1). The organism does not form spores in living tissue; sporulation...
Anthrax occurs only after the infected body has been opened and exposed to oxygen. The spores, which cause no swelling of the bacilli, are oval and occur centrally or paracentrally (Figure 22-2). They are very resistant and may survive in the environment for decades in certain soil conditions. Bacterial identification is confirmed by demonstration of the protective antigen toxin component, lysis by a specific bacteriophage, detection of capsule by fluorescent antibody, and virulence for mice and guinea pigs. Additional confirmatory tests to identify toxin and capsule genes by the polymerase chain reaction have also been developed as research tools.

**Epidemiology**

Anthrax occurs worldwide. The organism exists in the soil as a spore. The question remains unsettled as to whether its persistence in the soil is due to significant multiplication of the organism in the soil, or if it is due solely to cycles of bacterial amplification in infected animals whose carcasses then contaminate the soil. The form of the organism in infected animals is the bacillus. Only when the organism in the carcass is exposed to air does sporulation occur.

Animals, domestic or wild, become infected when they ingest spores while grazing on contaminated land or eating contaminated feed. Environmental conditions such as drought, which may promote trauma in the oral cavity on grazing, are thought to increase the chances of acquiring anthrax, as Pasteur originally reported. Spread from animal to animal by mechanical means—by the biting flies, and from one environmental site to another by the nonbiting flies and by vultures—has been suggested.

Anthrax in humans is associated with agricultural, horticultural, or industrial exposure to infected animals or contaminated animal products. In the less-developed countries, primarily in Africa, Asia, and the Middle East, disease occurs from contact with infected domesticated animals or contaminated animal products. This includes handling contaminated carcasses, hides, wool, hair, and bones; and ingesting contaminated meat. Cases associated with industrial exposure, rarely seen today, occur in workers processing contaminated hair, wool, hides, and bones. Direct contact with contaminated material leads to cutaneous disease, while ingestion of infected meat gives rise to oropharyngeal or gastrointestinal forms of anthrax. Inhalation of a sufficient quantity of spores, usually seen only during generation of aerosols in an enclosed space associated with processing contaminated wool or hair, gives rise to inhalational anthrax.

Unreliable reporting makes it difficult to estimate with accuracy the true incidence of human anthrax. It was estimated in 1958 that worldwide between 20,000 and 100,000 cases occurred annually. In more recent years, anthrax in animals has been reported from 82 countries, and human cases continue to be reported from Africa, Asia, Europe, and the Americas. In the United States, the annual incidence of human anthrax has steadily declined—from about 127 cases in the early years of this century to about one per year for the past 10 years. The vast majority of cases have been cutaneous. Under natural conditions, inhalational anthrax is exceedingly rare, with only 18 cases having been reported in the United States in the 20th century. In the early years of this century, cases of inhalational anthrax were reported in rural villagers in Russia who worked with contaminated sheep wool inside their homes. Five cases of inhalational anthrax occurred in woolen mill workers in New Hampshire in the 1950s. During times of economic hardship and disruption of veterinary and human public health practices, such as occurs during war, there have been large epidemics of anthrax. The largest reported epidemic of human anthrax occurred in Zimbabwe from 1978 through 1980, with an estimated 10,000 cases. Essentially all were cutaneous, with very rare cases of gastrointestinal disease and eight cases of inhalational anthrax, although no autopsy confirmation was reported.

**Pathogenesis**

*B. anthracis* possesses three known virulence factors: an antiphagocytic capsule and two protein exotoxins, called the *lethal* and the *edema* toxins. The role of the capsule in pathogenesis was demonstrated in the early 1900s, when anthrax strains lacking a capsule were shown to be avirulent. In more recent years, the genes encoding synthesis of the capsule were found to be encoded on a 110-kilobase (kb) plasmid. Molecular analysis revealed that strains cured of this plasmid no longer produced the capsule and were attenuated, thus confirming the critical role of the capsule in virulence. The capsule is composed of a polymer of poly-D-glutamic acid, which confers resistance to phagocytosis and
may contribute to the resistance of anthrax to lysis by serum cationic proteins.\(^{19}\)

It was Koch, in his initial studies on anthrax, who first suggested the importance of toxins. In 1954, Smith and Keppie\(^ {20}\) demonstrated a toxic factor in the serum of infected animals that was lethal when injected into other animals. The role of toxins in virulence and immunity was firmly established by many workers in the ensuing years.\(^ {21-23}\) Advances in molecular biology in the last decade have produced a more complete understanding of the biochemical mechanisms of action of the toxins and have begun to provide a more definitive picture of their role in the pathogenesis of the disease.

The genes encoding the synthesis of the two protein exotoxins are located on a 60-kb plasmid, distinct from that encoding for the capsule. In an environment of increased bicarbonate and carbon dioxide and increased temperature, such as is found in the infected host, there is increased transcription of the genes for synthesis of the two toxins,\(^ {24-26}\) as well as for the capsule.\(^ {27}\)

The anthrax toxins, like many bacterial and plant toxins, possess two components: a cell-binding, or B, domain; and an active, or A, domain that has the toxic and, usually, the enzymatic activity (Figure 22-3). The B and A anthrax toxin components are synthesized from different genes and are secreted as noncovalently linked proteins. The two toxins are unusual in that the B protein, called protective antigen (MW 83,000), is shared by both toxins. Thus the lethal toxin is composed of the protective antigen combined with a second protein, which is known as the lethal factor (MW 90,000). The lethal toxin is lethal for experimental animals\(^ {28,29}\) and the lethal factor has been shown to possess homology to metalloproteases, although no direct enzymatic activity has yet been discovered.\(^ {30}\)

The edema toxin, consisting of the same protective antigen together with a third protein, edema factor (MW 89,000), causes edema when injected into the skin of experimental animals.\(^ {28,29}\) The edema factor is a calmodulin-dependent adenylate cyclase, which elevates intracellular cyclic adenosine monophosphate, and which is likely to be responsible for the marked edema often present at the site of bacterial replication.

Each of the three toxin proteins—the B protein and both A proteins—individually is without biological activity. The critical role of the toxins in pathogenesis was established when it was shown that deletion of the toxin encoding plasmid\(^ {18,31}\) or the protective antigen gene alone\(^ {32}\) attenuates the organism. The lethal toxin also appears to be more important for virulence in a mouse model than the edema toxin.\(^ {33}\)

Recent studies in cell culture models have given a clearer understanding of the molecular interactions of the toxin proteins. Protective antigen first binds, most likely by a domain at its carboxy-terminus,\(^ {34,35}\) to a specific cell receptor.\(^ {36}\) Once bound, it is cleaved by a protease located on the cell surface,\(^ {37,38}\) resulting in retention on the cell surface of a 63-kilodalton (kd) fragment of protective antigen. This cleavage creates a binding site on the protective antigen to which either the lethal factor or the edema factor can bind with high affinity. The complex is then internalized and passes through an acidic vesicle and is translocated to the cell cytosol, where it expresses its toxic activity.

The situation in the infected animal may be somewhat different, since the toxin proteins may exist in the serum as a complex of protective antigen and lethal factor.\(^ {39}\) It is possible that the proteolytic activation of protective antigen necessary to form lethal or edema toxin may occur in interstitial fluid or serum rather than on the cell surface. The lethal or the edema toxin may then bind to target cells and be internalized.

Infection begins when the spores are inoculated through the skin or mucosa. It is thought that spores are ingested at the local site by macrophages, in which they germinate to the vegetative bacillus with production of capsule and toxins. At these sites, the
Anthrax

Once in the tracheobronchial lymph nodes, the local production of toxins by extracellular bacilli gives rise to the characteristic pathological picture: massive hemorrhagic, edematous, and necrotizing lymphadenitis; and mediastinitis (the latter is almost pathognomonic of this disease). The bacilli can then spread to the blood, leading to septicemia with seeding of other organs and frequently causing hemorrhagic meningitis. Terminally, toxin is present in high concentrations in the blood, but both the site of toxin action and the molecular mechanism of death remain unknown. Death is the result of respiratory failure associated with pulmonary edema, overwhelming bacteremia, and, often, meningitis.

Crude toxin preparations have been shown to impair neutrophil chemotaxis, phagocytosis, and killing. More recent work has shown that purified edema toxin impairs phagocytosis and priming for the respiratory burst in neutrophils, and also inhibits the production of interleukin-6 (IL-6) and tumor necrosis factor (TNF) by monocytes, which may further impair host resistance. The lethal toxin is directly cytolytic for macrophages, causing release of the potentially toxic cytokines IL-1 and TNF. Experimentally, animals can be protected against death from lethal toxin by depleting them of macrophages or blocking the effect of IL-1, but the role of these cytokines in death from infection remains to be established.

CLINICAL DISEASE

The military’s interest in anthrax is with defense against its use as an inhalational biological weapon. However, other forms of the disease are far more likely to be seen by medical officers—particularly when deployed to Third World countries—and are therefore included for completeness.

Cutaneous Anthrax

More than 95% of cases of anthrax are cutaneous (Figure 22-4). After inoculation, the incubation period is 1 to 5 days. The disease first appears as a small papule that progresses over a day or two to a vesicle containing serosanguinous fluid with many organisms and a paucity of leukocytes. The vesicle, which may be 1 to 2 cm in diameter, ruptures, leaving a necrotic ulcer. Satellite vesicles may also be present. The lesion is usually painless, and varying degrees of edema may be present around it. The edema may occasionally be massive, encompassing the entire face or limb, and is described by the term “malignant edema.” Patients usually have fever,
malaise, and headache, which may be severe in those with extensive edema. There may also be local lymphadenitis. The ulcer base develops a characteristic black eschar and after a period of 2 to 3 weeks the eschar separates, often leaving a scar. Septicemia is very rare, and with treatment mortality should be less than 1%.

Inhalational Anthrax

Inhalational anthrax begins after an incubation period of 1 to 6 days with nonspecific symptoms of malaise, fatigue, myalgia, and fever. There may be an associated nonproductive cough and mild chest discomfort. These symptoms usually persist for 2 or 3 days, and in some cases there may be a short period of improvement. This is followed by the sudden onset of increasing respiratory distress with dyspnea, stridor, cyanosis, increased chest pain, and diaphoresis. There may be associated edema of the chest and neck. Chest X-ray examination usually shows the characteristic widening of the mediastinum and, often, pleural effusions (Figure 22-5). Pneumonia has not been a consistent finding but can occur in some patients.\(^5\) While cases of inhalational anthrax have been rare in this century, several have occurred in patients with underlying pulmonary disease, suggesting that this condition may increase susceptibility to the disease.\(^3\) Meningitis is present in up to 50% of cases, and some patients may present with seizures. The onset of respiratory distress is followed by the rapid onset of shock and death within 24 to 36 hours. Mortality has been essentially 100% despite appropriate treatment.

Oropharyngeal and Gastrointestinal Anthrax

Oropharyngeal and gastrointestinal anthrax result from the ingestion of infected meat that has not been sufficiently cooked. After an incubation period of 2 to 5 days, patients with oropharyngeal disease present with severe sore throat or a local oral or tonsillar ulcer, usually associated with fever, toxicity, and swelling of the neck due to cervical or submandibular lymphadenitis and edema. Dysphagia and respiratory distress may also be present. Gastrointestinal anthrax begins with nonspecific symptoms of nausea, vomiting, and fever; these are followed in most cases by severe abdominal pain. The presenting sign may be an acute abdomen, which may be associated with hematemesis, massive ascites, and diarrhea. Mortality in both forms may be as high as 50%, especially in the gastrointestinal form.

Meningitis

Meningitis may occur following bacteremia, as a complication of any of the other clinical forms of the disease. Meningitis may also occur, very rarely, without a clinically apparent primary focus. It is very often hemorrhagic, which is important diagnostically, and almost invariably fatal (Figure 22-6).
DIAGNOSIS

The most critical aspect in making a diagnosis of anthrax is a high index of suspicion associated with a compatible history of exposure. Cutaneous anthrax should be considered following the development of a painless pruritic papule, vesicle, or ulcer—often with surrounding edema—that develops into a black eschar. With extensive or massive edema, such a lesion is almost pathognomonic. Gram’s stain or culture of the lesion will usually confirm the diagnosis. The differential diagnosis should include tularemia, staphylococcal or streptococcal disease, and orf (a viral disease of sheep and goats, transmissible to humans).

The diagnosis of inhalational anthrax is extraordinarily difficult, but the disease should be suspected with a history of exposure to a Bacillus anthracis-containing aerosol. The early symptoms are entirely nonspecific. However, (1) the development of respiratory distress in association with radiographic evidence of a widened mediastinus due to hemorrhagic mediastinitis, and (2) the presence of hemorrhagic pleural effusion or hemorrhagic meningitis should suggest the diagnosis. Sputum examination is not helpful in making the diagnosis, since pneumonia is not usually a feature of inhalational anthrax.

Gastrointestinal anthrax is exceedingly difficult to diagnose because of the rarity of the disease and its nonspecific symptoms. Only with a history of ingesting contaminated meat in the setting of an outbreak is diagnosis usually considered. Microbiologic cultures are not helpful in confirming the diagnosis. The diagnosis of oropharyngeal anthrax can be made from the clinical and physical findings in a patient with the appropriate epidemiological history.

Meningitis due to anthrax is clinically indistinguishable from meningitis due to other etiologies. An important distinguishing feature is that the cerebral spinal fluid is hemorrhagic in as many as 50% of cases. The diagnosis can be confirmed by identifying the organism in cerebral spinal fluid by microscopy, culture, or both.

Serology is generally only of use in making a retrospective diagnosis. Antibody to protective antigen or the capsule develops in 68% to 93% of reported cases of cutaneous anthrax and 67% to 94% of reported cases of inhalational anthrax. A positive skin test to anthraxin (an undefined antigen derived from acid hydrolysis of the bacillus that was developed and evaluated in the former Soviet Union) has also been reported to be of value in the retrospective diagnosis of anthrax. Western countries have limited experience with this test.

TREATMENT

Penicillin is the drug of choice for anthrax. Cutaneous anthrax without toxicity or systemic symptoms may be treated with oral penicillin. If evidence of spreading infection or systemic symptoms is present, then intravenous therapy with high-dose penicillin (2 million units administered every 6 h) may be initiated until a clinical response is obtained. Effective therapy will reduce edema and systemic symptoms but will not change the evolution of the skin lesion itself. Treatment should be continued for 7 to 10 days.

Tetracycline, erythromycin, and chloramphenicol have also been used successfully. These drugs may be used for treatment of the rare case caused by naturally occurring penicillin-resistant organisms. Additional antibiotics shown to be active in vitro include ciprofloxacin, gentamicin, cefazolin, cephalothin, vancomycin, clindamycin, and imipenem. These drugs should be effective in vivo, but there is no reported clinical experience.

Inhalational, oropharyngeal, and gastrointestinal anthrax should be treated with large doses of intravenous penicillin (2 million units administered every 2 h) with appropriate vasopressors, oxygen, and other supportive therapy.

PROPHYLAXIS

Prophylactic Treatment After Exposure

Experimental evidence has demonstrated that treatment with antibiotics beginning 1 day after exposure to a lethal aerosol challenge with anthrax spores can provide significant protection against death. All three drugs used in this study—ciprofloxacin, doxycycline, and penicillin—were effective. The optimal protection was afforded by combining antibiotics with active immunization.

Active Immunization

The only licensed human vaccine against anthrax is produced by the Michigan Department of Public Health. This vaccine is made from sterile filtrates...
of microaerophilic cultures of an attenuated, unencapsulated, nonproteolytic strain (V770-NP1-R) of B anthracis. The filtrate, containing predominantly protective antigen, is adsorbed to aluminum hydroxide. The final product also contains formaldehyde, in a final concentration of no more than 0.02%, and benzethonium chloride 0.0025%, as preservatives. Some vaccine lots contain very small amounts of lethal factor and lesser amounts of edema factor, as determined by antibody responses in vaccinated animals,18,59,60 although this antibody response has not been reported in the limited observations in human vaccinees.61 Although protective antigen by itself is an effective immunogen,62 it is unknown whether the small amounts of lethal or edema factor that are present in some lots of the vaccine contribute to its protective efficacy. The potency of vaccine lots is determined by showing protection of parenterally challenged guinea pigs. There is no characterization of the amount and form of the protective antigen or other toxin components in the vaccine. The vaccine is stored at 2°C to 8°C. The recommended schedule for vaccination is 0.5 mL given subcutaneously at 0, 2, and 4 weeks, followed by boosters of 0.5 mL at 6, 12, and 18 months. Annual boosters are recommended if the potential for exposure continues.

The vaccine should be given to industrial workers exposed to potentially contaminated animal products imported from countries in which animal anthrax remains uncontrolled. These products include wool, goat hair, hides, and bones. People in direct contact with potentially infected animals as well as laboratory workers should also be immunized. Vaccination is also indicated for protection against the use of anthrax in biological warfare. Approximately 150,000 service members received this licensed MDPH vaccine between 11 January and 28 February 1991 (25%–30% of the total U.S. forces deployed during the Persian Gulf War).

A live, attenuated, unencapsulated, spore vaccine is used for humans in the former USSR. The vaccine is given by scarification or subcutaneously. Its developers claim it to be reasonably well tolerated and to show some degree of protective efficacy against cutaneous anthrax in clinical field trials.53

In the United States, immunization with the licensed vaccine induced an immune response, measured by indirect hemagglutination, to protective antigen in 83% of vaccinees 2 weeks after the first three doses,63 and in 91% of those tested after receiving two or more doses.60 One hundred percent of the vaccinees develop a rise in titer in response to the yearly booster dose. When tested by an enzyme-linked immunosorbent assay, the current serological test of choice, more than 95% of vaccinees seroconvert after the initial three doses.51,64

A rough correlation between antibody titer to protective antigen and protection of experimental animals from infection exists after vaccination with the human vaccine. However, the exact relationship between antibody to protective antigen as measured in these assays, and immunity to infection remains obscure because the live, attenuated Sterne veterinary vaccine (made from an unencapsulated, toxin-producing strain) protects animals better than the human vaccine, yet it induces lower levels of antibody to protective antigen.59–61

The protective efficacy of experimental protective antigen-based vaccines produced from sterile culture filtrates of B anthracis was clearly demonstrated using various animal models and routes of challenge.21,65 A placebo-controlled clinical trial was conducted with a vaccine similar to the currently licensed U.S. vaccine.66 This field-tested vaccine was composed of the sterile, cell-free culture supernatant from an attenuated, unencapsulated strain of B anthracis—different from that used to produce the licensed vaccine and grown under aerobic, rather than microaerophilic, conditions.67 It was precipitated with alum rather than adsorbed to aluminum hydroxide. The study population worked in four mills in the northeastern United States where B anthracis–contaminated imported goat hair was used. The vaccinated group, compared to a placebo-inoculated control group, was afforded 92.5% protection against cutaneous anthrax, with a lower 95% confidence limit of 65% effectiveness. There were insufficient cases of inhalational anthrax to determine whether the vaccine was effective against this form of the disease. This same vaccine was previously shown to protect rhesus monkeys against an aerosol exposure to anthrax spores.67

There have been no controlled clinical trials in humans of the efficacy of the currently licensed U.S. vaccine. This vaccine has been extensively tested in animals and has protected guinea pigs against both an intramuscular60,61 and an aerosol challenge.59 The licensed vaccine has also been shown to protect rhesus monkeys against an aerosol challenge.58,68

**Side Effects**

In two different studies, the incidence of significant local and systemic reactions to the vaccine used in the placebo-controlled field trial was 2.4% to 2.8%66 and 0.2% to 1.3%.67 The vaccine currently
Anthrax is a zoonotic disease that occurs in domesticated and wild animals. Humans become infected by contact with infected animals or contaminated products. Under natural circumstances, infection occurs by the cutaneous route and only extremely rarely by the inhalational or gastrointestinal routes.

An aerosol exposure to spores causes inhalational anthrax. This form of the disease, which is of military concern because of its potential for use as a biological warfare agent, begins with nonspecific symptoms followed in 2 to 3 days by the sudden onset of respiratory distress with dyspnea, cyanosis, and stridor. It is rapidly fatal. Radiographic examination of the chest often reveals the characteristic mediastinal widening, indicative of hemorrhagic mediastinitis. Hemorrhagic meningitis frequently coexists. Given the rarity of the disease and its rapid progression, the diagnosis of inhalational anthrax is difficult to make. Treatment consists of massive doses of antibiotics and supportive care. Postexposure antibiotic prophylaxis is effective in experimental animals and should be instituted as soon as possible after exposure. A licensed nonliving vaccine is available for human use.

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