Review Article

Medical Progress

ANTHRAX

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ANTHRAX is an often fatal bacterial infection that occurs when *Bacillus anthracis* endospores enter the body through abrasions in the skin or by inhalation or ingestion.[1] It is a zoonosis to which most mammals, especially grazing herbivores, are considered susceptible. Human infections result from contact with contaminated animals or animal products, and there are no known cases of human-to-human transmission. Human anthrax is not common, and only one of us has seen a case. Cutaneous anthrax, the most common form, is usually curable. A small percentage of cutaneous infections become systemic, and these can be fatal. Systemic infection resulting from inhalation of the organism has a mortality rate approaching 100 percent, with death usually occurring within a few days after the onset of symptoms.[2] The rate of mortality among persons with infection resulting from ingestion is variable, depending on the outbreak, but it may also approach 100 percent. Whatever the portal of entry, systemic anthrax involves massive bacteremia and toxemia with nondescript initial symptoms until the onset of hypotension, shock, and sudden death. Manifestations of advanced disease, including shock and sudden death, are believed to result from the action of the exotoxin complex secreted by anthrax bacilli.[1,3] The efficacy of therapy, if initiated during the incubation period, and the rapid course of the disease once symptoms appear make early intervention an absolute necessity. Inglesby et al. have provided a description of the policies and strategies for dealing with anthrax as a biologic weapon.[4] The goal of this article is to familiarize physicians with the current understanding of the pathogenesis, diagnosis, prevention, and treatment of anthrax.

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PATHOGENESIS

Anthrax infections are initiated by endospores of *B. anthracis,* a gram-positive soil organism. Anthrax endospores do not divide, have no measurable metabolism, and are resistant to drying, heat, ultraviolet light, gamma radiation, and many disinfectants.[5] In some types of soil, anthrax spores can remain dormant for decades. Their hardiness and dormancy have allowed anthrax spores to be developed as biologic weapons by a number of nations, although their only known use in war was by the Japanese army in Manchuria in the 1940s.[6] All known anthrax virulence genes are expressed by the vegetative form of *B. anthracis* that results from the germination of spores within the body.

The course of infection and clinical manifestations are depicted in Figure 1. Endospores introduced into the body by abrasion, inhalation, or ingestion are phagocytosed by macrophages and carried to regional lymph nodes. Endospores germinate inside the macrophages and become vegetative bacteria[7,8]; the vegetative bacteria are then released from the macrophages, multiply in the lymphatic system, and enter the bloodstream, until there are as many as 10^7 to 10^8 organisms per milliliter of blood, causing massive septicemia. Once they have been released from the macrophages, there is no evidence that an immune response is initiated against vegetative bacilli. Anthrax bacilli express virulence factors, including toxin and capsule.[1] The resulting toxemia has systemic effects that lead to the death of the host.

The major virulence factors of *B. anthracis* are encoded on two virulence plasmids, pXO1 and pXO2. The toxin-bearing plasmid, pXO1, is 184.5 kilobase pairs (kbp) in size and codes for the genes that make up the secreted exotoxins. The toxin-gene complex is composed of protective antigen, lethal factor, and edema factor.[9] The three exotoxin components combine to form two binary toxins. Edema toxin consists of edema factor, which is a calmodulin-dependent adenylate cyclase,[10,11] and protective antigen, the binding moiety that permits entry of the toxin into the host cell. Increased cellular levels of cyclic AMP upset water homeostasis and are believed to be responsible for the massive edema seen in cutaneous anthrax. Edema toxin inhibits neutrophil function in vitro,[12] and neutrophil function is impaired in patients with

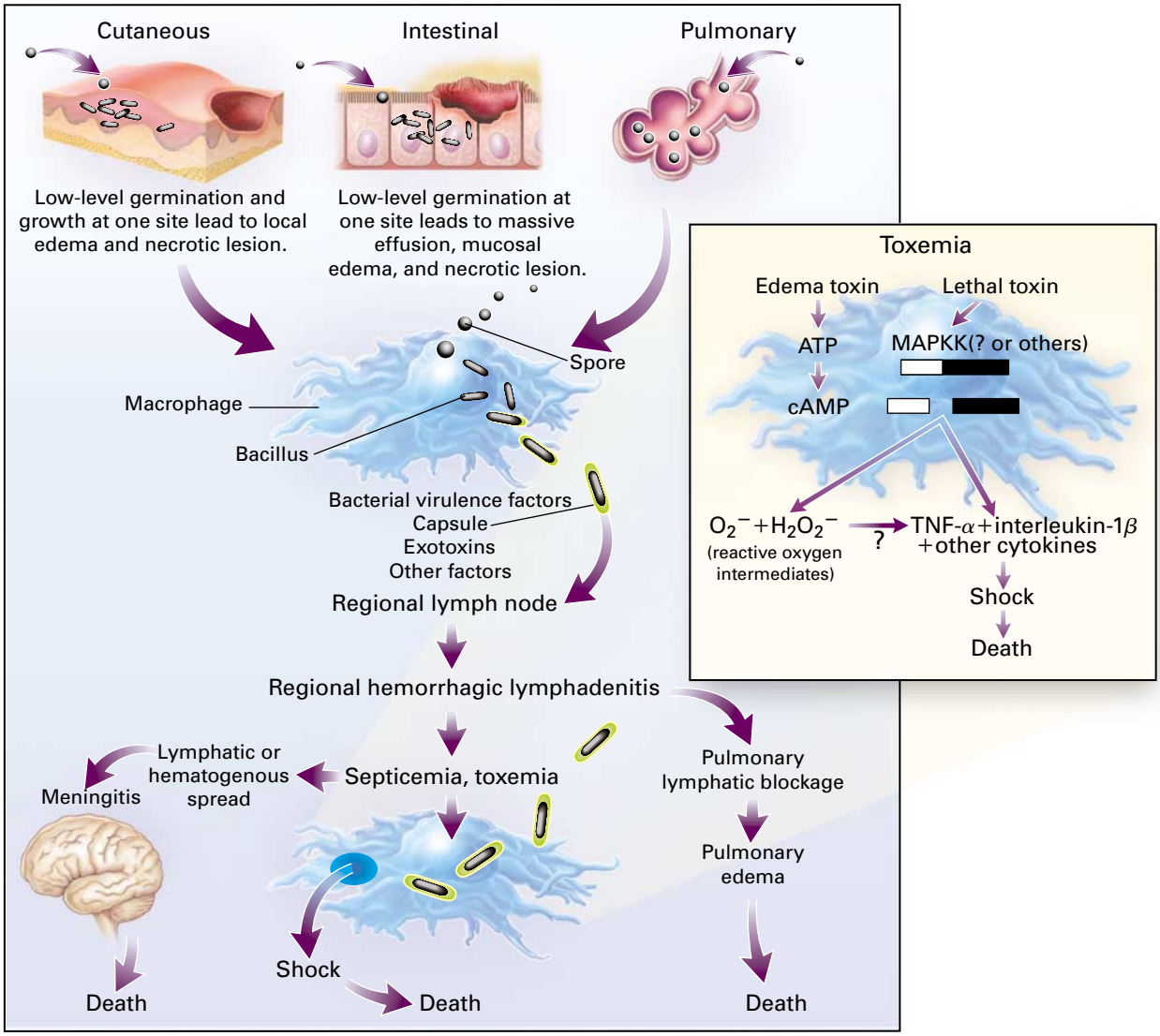


Figure 1. Pathophysiology of Anthrax.

Pathogenic *Bacillus anthracis* endospores reach a primary site in the subcutaneous layer, gastrointestinal mucosa, or alveolar spaces. For cutaneous and gastrointestinal anthrax, low-level germination occurs at the primary site, leading to local edema and necrosis. Endospores are phagocytosed by macrophages and germinate. Macrophages containing bacilli detach and migrate to the regional lymph node. Vegetative anthrax bacilli grow in the lymph node, creating regional hemorrhagic lymphadenitis. Bacteria spread through the blood and lymph and increase to high numbers, causing severe septicemia. High levels of exotoxins are produced that are responsible for overt symptoms and death. In a small number of cases, systemic anthrax can lead to meningeal involvement by means of lymphatic or hematogenous spread. In cases of pulmonary anthrax, peribronchial hemorrhagic lymphadenitis blocks pulmonary lymphatic drainage, leading to pulmonary edema. Death results from septicemia, toxemia, or pulmonary complications and can occur one to seven days after exposure.

The inset shows the effects of anthrax exotoxins on macrophages. Vegetative anthrax bacilli secrete two exotoxins that are active in host cells. Edema toxin is a calmodulin-dependent adenylate cyclase that increases intracellular levels of cyclic AMP (cAMP) on entry into most types of cell. This is believed to alter water homeostasis, resulting in massive edema. Lethal toxin is a zinc metalloprotease that causes a hyperinflammatory condition in macrophages, activating the oxidative burst pathway and the release of reactive oxygen intermediates, as well as the production of proinflammatory cytokines, such as tumor necrosis factor α (TNF-α) and interleukin-1β, that are responsible for shock and death. MAPKK denotes mitogen-activated protein kinase kinase.

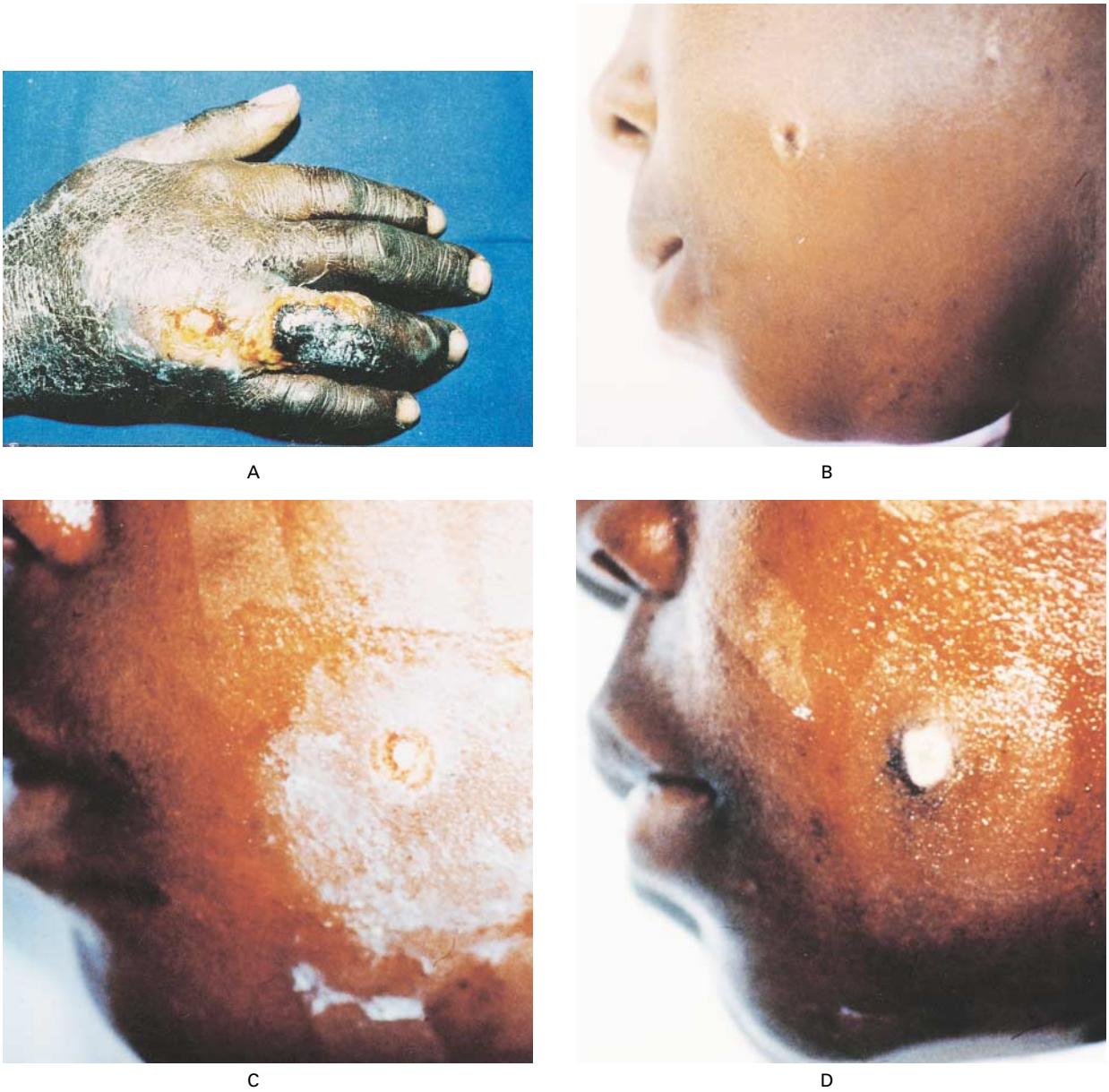


Figure 2. Cutaneous Anthrax Infection of the Hand and Cheek.

Panel A shows the characteristic blackened eschar surrounded by eroded areas and massive edema. These lesions are painless. The areas of "dried skin" represent resolving edema. Lesions continue to progress despite rigorous antibiotic treatment. Cutaneous anthrax can be self-limiting, and the lesions resolve without scarring. About 10 percent of untreated cutaneous anthrax infections progress to systemic anthrax. Panels B, C, and D show changes in the lesion on the cheek over a seven-day period. The characteristic blackened eschar is present on day 0 (Panel B). Facial edema and ulceration occur by the second day (Panel C). On day 7, the lesion is beginning to heal, and the facial edema is resolving (Panel D). The photograph in Panel A was kindly provided by Drs. Wilhelm Kobuch and P.C.B. Turnbull. The photographs in Panels B, C, and D are reprinted from Smego et al.[33] with the permission of the publisher.

cutaneous anthrax infection.[13] Lethal toxin consists of lethal factor, which is a zinc metalloprotease[14-16] that inactivates mitogen-activated protein kinase kinase in vitro,[17,18] and protective antigen, which acts as the binding domain. Lethal toxin stimulates the macrophages to release tumor necrosis factor α and interleukin-1β, which are partly responsible for sudden death in systemic anthrax (Fig. 1, inset).[3,15,19]

The smaller capsule-bearing plasmid, pXO2, is 95.3 kbp in size and codes for three genes (*capB*, *capC*, and *capA*) involved in the synthesis of the polyglutamyl capsule.[20] The exotoxins are thought to

inhibit the immune response mounted against infection, whereas the capsule inhibits phagocytosis of vegetative anthrax bacilli. The expression of all known major virulence factors is regulated by host-specific factors such as elevated temperature (≥37°C) and carbon dioxide concentration (≥5 percent), and by the presence of serum components.[21,22] Regulation of the expression of the toxin and capsule genes is mediated by the transcriptional activator AtxA, whose activity appears to be affected by the previously mentioned environmental conditions.[23-25] Expression of the capsule gene is also controlled by its own transcriptional regulator, AcpA.[26] Both plasmids are required for full virulence; the loss of either one results in an attenuated strain. Historically, bacterial strains for anthrax vaccine were made by rendering virulent strains free of one or both plasmids. Pasteur, an avirulent pXO2-carrying strain, is encapsulated but does not express exotoxin components.[1] Sterne, an attenuated strain that carries pXO1, can synthesize exotoxin components but does not have a capsule.[1]

CLINICAL MANIFESTATIONS

Cutaneous Anthrax

Cutaneous anthrax accounts for 95 percent of all anthrax infections in the United States.[27-30] The name anthrax (from the Greek for coal) refers to the typical black eschar that is seen on affected areas (Fig. 2). Patients often have a history of occupational contact with animals or animal products. The most common areas of exposure are the head, neck, and extremities, although any area can be involved. Pathogenic endospores are introduced subcutaneously through a cut or abrasion. There are a few case reports of transmission by insect bites, presumably after the insect fed on an infected carcass.[31,32] The primary skin lesion is usually a nondescript, painless, pruritic papule that appears three to five days after the introduction of endospores. In 24 to 36 hours, the lesion forms a vesicle that undergoes central necrosis and drying, leaving a characteristic black eschar surrounded by edema and a number of purplish vesicles. The edema is usually more extensive on the head or neck than on the trunk or extremities.[33] The common description "malignant pustule" is actually a misnomer, because the cutaneous lesion is not purulent and is characteristically painless. A painful, pustular eschar in a febrile patient indicates a secondary infection, most often with staphylococcus or streptococcus.[34]

Although cutaneous anthrax can be self-limiting, antibiotic treatment is recommended. Lesions resolve without complications or scarring in 80 to 90 percent of cases. Malignant edema is a rare complication characterized by severe edema, induration, multiple bullae, and symptoms of shock.[35,36] Malignant edema involving the neck and thoracic region often leads to breathing difficulties that require corticosteroid therapy or intubation. A few cases have been reported of temporal arteritis associated with cutaneous anthrax infection and of corneal scarring from palpebral cutaneous anthrax.[37,38] Histologic examination of anthrax skin lesions shows necrosis and massive edema with lymphocytic infiltrates. There is no liquefaction or abscess formation, indicating that the lesions are not suppurative. Focal points of hemorrhage are evident, with some thrombosis.[39] Gram's staining reveals bacilli in the subcutaneous tissue.[39]

Gastrointestinal and Oropharyngeal Anthrax

Gastrointestinal anthrax, which can be fatal, has not been reported in the United States. The symptoms appear two to five days after the ingestion of endospore-contaminated meat from diseased animals.[40] Therefore, multiple cases can occur within individual households.[40,41] An unusually prolonged outbreak was attributed to the consumption of stored meat products.[42] It is presumed that bacterial inoculation takes place at a breach in the mucosal lining, but exactly where the endospores germinate is unknown. On pathological examination, bacilli can be seen microscopically in the mucosal and submucosal lymphatic tissue, and there is gross evidence of mesenteric lymphadenitis.[43] Ulceration is always seen. It is not known whether ulceration occurs only at sites of bacterial infection or is distributed more diffusely as a result of the action of anthrax toxin.[43-45] Microscopical examination of affected tissues reveals massive edema and mucosal necrosis at infected sites.[45] Inflammatory infiltrates are seen that are similar to those in cutaneous anthrax. Gram's staining of peritoneal fluid may reveal numerous large gram-positive bacilli.[40,46]

Although mediastinal widening is considered pathognomonic of inhalational anthrax, it has also been reported in a case of gastrointestinal anthrax.[47] Associated symptoms include fever and diffuse abdominal pain with rebound tenderness. There are reports of both constipation and diarrhea; the stools are either melenic or blood-tinged.[46,48] Because of ulceration of the gastrointestinal mucosa, patients often vomit material that is blood-tinged or has a coffee-ground appearance. Ascites develops with concomitant reduction in abdominal pain two to four days after the onset of symptoms. The appearance of the ascites fluid ranges from clear to purulent, and it often yields colonies of *B. anthracis* when cultured. Morbidity is due to blood loss, fluid and electrolyte imbalances, and subsequent shock. Death results from intestinal perforation or anthrax toxemia. If the patient survives, most of the symptoms subside in 10 to 14 days.[48]

Oropharyngeal anthrax is less common than the gastrointestinal form. It is also associated with the ingestion of contaminated meat. Initial symptoms include cervical edema and local lymphadenopathy, which cause dysphagia and respiratory difficulties. Lesions can be seen in the oropharynx and usually

have the appearance of pseudomembranous ulcerations. This form is milder than the classic gastrointestinal disease and has a more favorable prognosis.[34,48]

Inhalational Anthrax

Inhalational anthrax is rare, usually occurring after the inhalation of pathogenic endospores from contaminated animal hides or products. Before the introduction of hygienic measures in the 1960s, including vaccination, workers in goat-hair mills, for example, were regularly exposed to high concentrations of viable anthrax spores. Nevertheless, for reasons that are not understood, few cases of inhalational anthrax occurred among them.[49-51] When dispersed in the atmosphere as an aerosol, anthrax spores can present a respiratory hazard even far downwind from the point of release, as demonstrated by animal tests on Gruinard Island in the United Kingdom,[52-55] and by an accidental release from a military biologic facility in the city of Sverdlovsk in the former Soviet Union.[2,56-58]

Inhalational anthrax is usually fatal, even with aggressive antimicrobial therapy. It appears that only about one fifth of those who contracted inhalational anthrax in Sverdlovsk recovered.[2] Anthrax spores are about 1 to 2 μm in diameter, a size that is optimal for inhalation and deposition in the alveolar spaces.[51,59-61] Although the lung is the initial site of contact, inhalational anthrax is not considered a true pneumonia. In most but not all cases, there is no infection in the lungs.[58,62] Rather, the endospores are engulfed by alveolar macrophages and transported by them to the mediastinal and peribronchial lymph nodes, with the spores germinating en route. Anthrax bacilli multiply in the lymph nodes, causing hemorrhagic mediastinitis, and spread throughout the body in the blood.[43,62]

Data from the Sverdlovsk outbreak indicate a modal incubation time of approximately 10 days for inhalational anthrax. However, the onset of symptoms occurred up to six weeks after the reported date of exposure.[2,57] Such long incubation times presumably reflect the ability of viable anthrax spores to remain in the lungs for many days.[51,63,64] Longer incubation periods may be associated with smaller inocula.

The initial symptoms most often reported are fever, nonproductive cough, myalgia, and malaise, resembling those of a viral upper respiratory tract infection. Early in the course of the disease, chest radiographs show a widened mediastinum, which is evidence of hemorrhagic mediastinitis, and marked pleural effusions. After one to three days, the disease takes a fulminant course with dyspnea, strident cough, and chills, culminating in death.[34,59] In Sverdlovsk, the mean time between the onset of symptoms and death was 3 days (range, 1 to 10). Although accompanying evidence of clinical signs of pneumonia in these cases is lacking, some of the autopsies from the Sverdlovsk outbreak showed a focus of necrotizing hemorrhagic pneumonitis, possibly at the portal of infection.[58] Submucosal hemorrhages occurred in the trachea and bronchi, with hemorrhage and necrosis of peribronchial lymph nodes. Hemorrhagic mediastinal lymph nodes represent the primary lesion; however, gastrointestinal and leptomeningeal lesions are the result of hematogenous spread.

There may be wide individual variation in susceptibility to inhalational anthrax, as suggested by experimental studies in nonhuman primates and by the absence of persons younger than 24 years among the 66 deaths reported in the Sverdlovsk outbreak.[2,51,57]

Anthrax Meningitis

Involvement of the meninges by *B. anthracis* is a rare complication of anthrax.[65] The most common portal of entry is the skin, from which the organisms can spread to the central nervous system by hematogenous or lymphatic routes. Anthrax meningitis also occurs in cases of pulmonary and gastrointestinal anthrax.[58,66] Anthrax meningitis is almost always fatal, with death occurring one to six days after the onset of illness, despite intensive antibiotic therapy. In the few cases in which patients have survived, antibiotic therapy was combined with the administration of antitoxin, prednisone, or both.[65,67] In addition to common meningeal symptoms and nuchal rigidity, the patient has fever, fatigue, myalgia, headache, nausea, vomiting, and sometimes agitation, seizures, and delirium. The initial signs are followed by rapid neurologic degeneration and death. The pathological findings are consistent with a hemorrhagic meningitis, with extensive edema, inflammatory infiltrates, and numerous gram-positive bacilli in the leptomeninges.[43,68] The cerebrospinal fluid is often bloody and contains many gram-positive bacilli.[69] Gross examination at autopsy finds extensive hemorrhage of the leptomeninges, which gives them a dark red appearance described as "cardinal's cap."[58]

DIAGNOSIS

Differential Diagnosis

Table 1 summarizes the differential diagnosis of anthrax. In cutaneous anthrax, the painless, blackened, necrotic eschar is limited to the late stages of the infection. The ulcerative eschar of cutaneous anthrax must be differentiated from other papular lesions that present with regional lymphadenopathy. If the lesion is purulent and the regional lymph nodes are palpable, staphylococcal lymphadenitis is the most likely cause, although cutaneous anthrax lesions can be superinfected with pyogenic bacteria.[70]

The initial symptoms of inhalational anthrax are nondescript or "flulike" and are similar to those of atypical pneumonia from other causes. The prognosis is improved if early treatment is implemented, so that a high level of suspicion is necessary if there is a chance of exposure to anthrax. The cardiopulmo-

TABLE 1. DIFFERENTIAL DIAGNOSIS OF CLINICAL MANIFESTATIONS OF ANTHRAX.

MANIFESTATION	DISEASE	CAUSATIVE ORGANISM (IF APPLICABLE)
Cutaneous anthrax	Ecthyma gangrenosum	*Pseudomonas aeruginosa*
	Rat-bite fever	*Streptobacillus moniliformis, Spirillum minor*
	Ulceroglandular tularemia	*Francisella tularensis*
	Plague	*Yersinia pestis*
	Glanders	*Pseudomonas pseudomallei*
	Rickettsialpox	*Rickettsia akari*
	Orf	Parapoxvirus
	Staphylococcal lymphadenitis	*Staphylococcus aureus*
	Cutaneous tuberculosis	*Myocbacterium tuberculosis*
	Leprosy	*Mycobacterium leprae*
	Buruli ulcer	*Mycobacterium ulcerans*
Gastrointestinal anthrax	Typhoid	*Salmonella typhi*
	Intestinal tularemia	*Francisella tularensis*
	Acute gastroenteritis	
	Peritonitis	
	Mechanical obstruction	
	Peptic or duodenal ulcer	
Inhalational anthrax	Acute bacterial mediastinitis	
	Mycoplasmal pneumonia	*Mycoplasma pneumoniae*
	Legionnaires' disease	*Legionella pneumophila*
	Psittacosis	*Chlamydia psittaci*
	Tularemia	*Francisella tularensis*
	Q fever	*Coxiella burnetii*
	Viral pneumonia	Influenzavirus, hantavirus, adenovirus, respiratory syncytial virus, cytomegalovirus, varicella–zoster virus
	Histoplasmosis (fibrous mediastinitis)	*Histoplasma capsulatum*
	Coccidioidomycosis	*Coccidioides immitis*
	Ruptured aortic aneurysm	
	Superior vena cava syndrome	
	Silicosis	
	Sarcoidosis	
Meningeal anthrax	Subarachnoid hemorrhage	
	Bacterial meningitis	
	Aseptic meningitis	

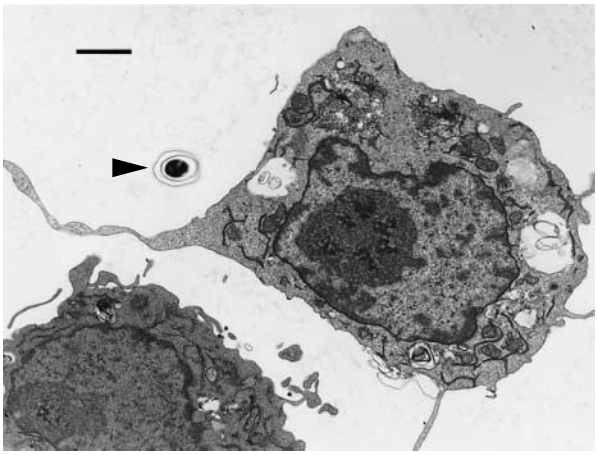
nary collapse associated with a history of radiographic evidence of mediastinal widening in the late stages of inhalational anthrax must be differentiated from cardiovascular collapse with noninfectious causes, such as dissecting or ruptured aortic aneurysm and the superior vena cava syndrome. Anthrax infection is unusual in that mediastinal changes can be detected early in the course of infection by chest radiography, although similar pictures can be seen in acute bacterial mediastinitis and fibrous mediastinitis due to *Histoplasma capsulatum*.[71] Less specific findings include pleural effusions and radiographic evidence of pulmonary edema. Silicosis, siderosis, alveolar proteinosis, and sarcoidosis are often alternative causes of chronic mediastinitis in patients with the relevant occupational history and previous chest radiographs demonstrating long-standing mediastinal widening.

When ingestion of contaminated meat is suspected, the symptoms of an acute abdomen should be considered as possible early signs of intestinal anthrax infection. Hemorrhagic meningitis caused by anthrax must be distinguished from subarachnoid hemorrhage by computed tomography without contrast. To distinguish hemorrhagic meningitis caused by *B. anthracis* from that caused by other bacteria, Gram's staining and culture of cerebrospinal fluid should be performed.[68] In addition to the above indictors, the clinician should consider anthrax if there is a history of contact with materials that may be contaminated with spores, such as infected farm animals and imported hides, or of travel to places where anthrax is endemic. Because of the remote possibility of an anthrax aerosol attack, clinicians should be alert to any sudden deaths of previously healthy persons from undiagnosed disease and report them promptly to the Centers for Disease Control and Prevention and other appropriate public health officials.

Bacteriologic Tests

B. anthracis is a nonmotile, gram-positive, aerobic rod 1.2 to 10 μm in length and 0.5 to 2.5 μm in width that is capable of forming central or terminal spores (Fig. 3).[72] It is part of the *B. cereus* group of bacilli, which consists of *B. cereus, B. anthracis, B. thurin-*

A



B

Figure 3. Photomicrographs of *Bacillus anthracis* Vegetative Cells and Spores.

Panel A shows a Gram's stain of *B. anthracis* vegetative bacteria. The bacterial cells exhibit gram-positive staining (purple filaments) (×600). Panel B shows an electron photomicrograph of a *B. anthracis* spore (arrowhead) partially surrounded by the pseudopod of a cultured macrophage (×137,000). The bar represents 1 μm.

giensis, and *B. mycoides.*[73] The bacteria in this group tend to be dismissed by clinical microbiology laboratories as contaminants unless the physician specifically requests testing.[73] Except for *B. anthracis,* all members of this group are resistant to penicillin because they produce chromosomally encoded beta-lactamases.[74] *B. anthracis* is easy to differentiate from other members of the *B. cereus* group by observing the morphologic features of the colony on a blood-agar plate. Colonies of most *B. anthracis* isolates are nonhemolytic and are white to gray, often looking like ground glass.[75] The unusually tenacious colonies are able to retain their shape when manipulated. When inoculated onto nutrient agar containing 0.7 percent bicarbonate and grown overnight at 37°C in the presence of 5 to 20 percent carbon dioxide, *B. anthracis* will form its characteristic poly-D-glutamic acid capsule.[76] These colonies have a mucoid appearance, and the capsule can be demonstrated microscopically in a colony smear stained with McFadyean's polychrome methylene blue or India ink.[75] Blood samples obtained from patients late in the course of infection and stained in the same manner will reveal large numbers of encapsulated bacilli. Bacilli can also be observed in and cultured from ascites fluid, pleural effusions, cerebrospinal fluid (in cases of meningitis),[77] and fluid carefully expressed from the eschar, although expressing eschar fluid is not recommended because it can cause dissemination of the pathogen.[78]

Patients with systemic disease often die before positive blood cultures can be obtained, making early diagnosis and treatment crucial. If the samples are likely to be contaminated with other bacillus species, polymyxin–lysozyme–EDTA–thallous acetate agar is used as a selective medium for *B. anthracis.*[79] The API 50 CH test strip (API Laboratory Products, Plainview, N.Y.) can be used in conjunction with the API 20E test strip to identify a number of bacillus species, including *B. anthracis.*[80] Blood cultures in cases of systemic anthrax infection are almost always positive, because of the large numbers of bacterial cells in the circulation.[1] Cultures of tissue from skin lesions, however, are not useful diagnostically, because the rate of positive cultures does not exceed 60 to 65 percent, probably owing to the use of antimicrobial therapy or the microbicidal activity of local antagonistic skin flora.[81] There are reports of clinical isolates of *B. anthracis* that are resistant to penicillin.[31,82] Because of the potential for drug-resistant strains, including deliberately modified strains, antibiotic-susceptibility testing should be performed on all isolates.

Serologic and Immunologic Tests

The major immunogenic proteins of *B. anthracis* appear to be capsular antigens and the exotoxin components. Specific enzyme-linked immunosorbent assays (ELISAs) that show a quadrupling of the titer of antibodies against these components are diagnostic of past infection or vaccination. The most reliable indicators are the titers of antibody to protective antigen and to capsular components.[73,83,84] In studies of the measurement of antibody titers by ELISA, the sensitivity of possible indicators was as follows: 72 percent for protective antigen, 95 to 100 percent for capsule antigens, 42 percent for lethal factor, and 26 percent for edema factor.[85] Enzyme-linked immunoelectrotransfer blotting provided a higher specificity when used in conjunction with ELISA-based testing.[85] Indirect microhemagglutination gives results similar to those obtained with ELISA but has certain drawbacks, including the short shelf life of an-

tigen-sensitized red-cell preparations, the limited reproducibility of the test, and longer preparation times.[86]

Immunologic detection of the exotoxins in blood during systemic infection is possible with similar tests if antibodies to anthrax toxins are available, but those tests are unreliable for diagnosis. Thus, although these tests are of epidemiologic value, they have little diagnostic value in acute illness.[83] During systemic infections, antibodies to toxin or capsular components cannot be detected until late in the course of the disease, often when it is too late to initiate treatment.[73] In treated infections, no increase in the antitoxin antibody titer is seen. The anthraxin skin test, consisting of subdermal injection of a commercially produced chemical extract of an attenuated strain of *B. anthracis*, is available for the diagnosis of acute and previous cases of anthrax.[81,87,88] In one study the skin test diagnosed 82 percent of cases one to three days after the onset of symptoms and 99 percent of cases by the end of the fourth week.[81] The skin test may be suitable for both rapid diagnosis of acute cases and the retrospective analysis of anthrax infections.

New Molecular Diagnostic Methods

New diagnostic techniques have focused on the use of the polymerase chain reaction to amplify markers specific to *B. anthracis* or the *B. cereus* group. Two markers, *vrrA*[89] and *Ba813*,[90-92] have been the subject of extensive study. Other methods using the polymerase chain reaction to amplify specific virulence plasmid markers harbored by different anthrax strains may soon become available.[56,93-96] These new rapid methods may become useful in the clinical setting, where early diagnosis is crucial.

PREVENTION AND TREATMENT

Prophylaxis, Vaccination, and Decontamination

Prophylaxis for asymptomatic patients with suspected exposure to anthrax spores can be achieved with a six-week course of doxycycline or ciprofloxacin. If the suspected dose of spores is high, a longer course of antibiotics is warranted. Extended treatment is needed for total pulmonary clearance of spores, which are not affected by the presence of antibiotics.[63,97]

The standard anthrax vaccine in the United States is approved by the Food and Drug Administration and is routinely administered to persons at risk for exposure to anthrax spores. The existing supplies are currently being used to immunize all military personnel. Designated "anthrax vaccine adsorbed" (AVA), it is an aluminum hydroxide–precipitated preparation of protective antigen from attenuated, nonencapsulated *B. anthracis* cultures of the Sterne strain.[98,99] Two inoculations with AVA afforded substantial protection against inhalational anthrax in rhesus monkeys,[100] and a limited trial of a similar vaccine in humans indicated that it afforded considerable protection against cutaneous anthrax.[101] AVA is administered subcutaneously in a 0.5-ml dose that is repeated at 2 and 4 weeks and at 6, 12, and 18 months.[102] Boosters are then given annually. For those receiving antibiotic prophylaxis for suspected exposure, AVA may be given concurrently. There is a need for vaccines with better protection and a simpler schedule. Vaccines now being tested include preparations of protective antigen subunits with different adjuvants, protective antigen purified from recombinant sources, and live vaccines based on anthrax strains with auxotrophic mutations.[103-113] Live attenuated endospore-based vaccines were widely used in the Soviet Union for both humans and livestock and remain in use in the Russian Federation today.[103] The ability of any vaccine to protect humans in the event of aerosol attack, as in biologic terrorism or warfare, cannot be tested directly and therefore must remain a concern.[114]

A textile mill contaminated with anthrax spores was decontaminated with vaporized formaldehyde,[115] and soil decontamination at Gruinard Island was achieved with formaldehyde in seawater.[116] Although decontamination is desirable, the risk that resuspension of a deposited aerosol will lead to inhalational anthrax is much less than the risk due to a primary aerosol.[117,118] Autoclaving and incineration are acceptable procedures for the decontamination of laboratory materials.

Treatment

Antibiotics

Table 2 summarizes pharmacologic therapy for anthrax. Penicillin and doxycycline are used for the treatment of anthrax. Intravenous administration is recommended in cases of inhalational, gastrointestinal, and meningeal anthrax. Cutaneous anthrax with signs of systemic involvement, extensive edema, or lesions on the head and neck also requires intravenous therapy. Streptomycin had a synergistic effect with penicillin in experiments and may also be given for inhalational anthrax. Despite early and vigorous treatment, the prognosis of patients with inhalational, gastrointestinal, or meningeal anthrax remains poor. Antibiotic therapy should be continued for at least 14 days after symptoms abate.[67,78] In cutaneous anthrax, treatment with oral penicillin renders lesions sterile after 24 hours, although they still progress to eschar formation. Chloramphenicol, erythromycin, tetracycline, or ciprofloxacin can be administered to patients who are allergic to penicillin. If resistance to penicillin and doxycycline is suspected and antibiotic-susceptibility data are not available, ciprofloxacin may be administered empirically. Doxycycline and tetracycline are not recommended for pregnant women or children, and the effects of ciprofloxacin in pregnant women have not been determined.[4]

For culturing cutaneous lesions, gentle sampling

TABLE 2. PHARMACOLOGIC THERAPY FOR *BACILLUS ANTHRACIS* INFECTION AND ITS SEQUELAE.*

THERAPY	DOSAGE FOR ADULTS	DOSAGE FOR CHILDREN
Treatment of infection†		
Penicillin V	200–500 mg orally 4 times/day	25–50 mg/kg of body weight/day orally in divided doses 2 or 4 times/day
Penicillin G	8 million–12 million U total, intravenously in divided doses every 4–6 hr	100,000–150,000 U/kg/day in divided doses every 4–6 hr
Streptomycin	30 mg/kg intramuscularly or intravenously per day — gentamicin can also be used (in conjunction with penicillin)	
Tetracycline	250–500 mg orally or intravenously 4 times/day	Tetracycline is not approved for children
Doxycycline	200 mg orally or intravenously as a loading dose, then 50–100 mg every 12 hr	Doxycycline is not approved for children <9 yr old For children ≤45 kg: 2.5 mg/kg every 12 hr For children >45 kg: use adult dosage
Erythromycin	250 mg orally every 6 hr	40/mg/kg/day orally in divided doses every 6 hr
Erythromycin lactobionate	15–20 mg/kg (maximum, 4 g) intravenously per day	20–40 mg/kg/day intravenously in divided doses every 6 hr (1- to 2-hr infusion)
Chloramphenicol	50–100 mg/kg/day orally or intravenously in divided doses every 6 hr	50–75 mg/kg/day in divided doses every 6 hr
Ciprofloxacin	250–750 mg orally twice/day 200–400 mg intravenously every 12 hr	20–30 mg/kg/day in divided doses every 12 hr Oral or intravenous dosing is not approved for patients <18 yr old
Prophylaxis‡		
Doxycycline	100 mg orally twice/day for 4 wk	
Ciprofloxacin	500 mg orally twice/day for 4 wk	
Corticosteroid therapy for severe edema		
Dexamethasone	0.75–0.90 mg/kg/day orally, intravenously, or intramuscularly in divided doses every 6 hr	0.25–0.5 mg/kg every 6 hr
Prednisone	1–2 mg/kg or 5–60 mg orally/day	0.5–2 mg/kg/day

*Most *B. anthracis* strains are resistant to cefuroxime in vitro.

†For inhalational, gastrointestinal, or meningeal anthrax infection in adults, the intravenous regimen is used with penicillin G, streptomycin, tetracycline, doxycycline, erythromycin lactobionate, chloramphenicol, and ciprofloxacin; for these infections in children, the intravenous regimen is used with penicillin G, doxycycline, erythromycin lactobionate, and chloramphenicol.

‡If patient is unvaccinated, begin initial doses of vaccine.

with a moist, sterile applicator is preferred. Excision of the eschar is contraindicated and might hasten systemic dissemination. Lesions should be covered with sterile dressings that are changed regularly. Soiled dressings should be autoclaved and properly disposed of. In cases of extensive edema, meningitis, or swelling in the head-and-neck region, corticosteroid therapy should be initiated.[119,120] Supportive therapy should be initiated to prevent septic shock and fluid and electrolyte imbalance, and to maintain airway patency.

Potential New Treatments

The current understanding that anthrax is a toxigenic condition suggests the potential of antitoxin therapy. The central importance of lethal toxin is supported by much research. Early experiments in which antibiotics were administered to animals at different stages of infection found a principle of "no return"; once the infection had reached a certain point, the animal was doomed, even after removal of the microbes. Test animals injected intravenously with purified lethal toxin died in a manner very similar to that of animals that died of the natural infection.[3,15,19] Lethal-toxin–deficient strains are highly attenuated.[121,122] Prior immunity (passive or active) to the lethal-toxin proteins protects animals from endospore challenge.[63,123] Finally, toxin-affected macrophages produce the proinflammatory cytokines that mediate the shock and sudden death that occur in anthrax.[3,15,19] Unfortunately, antitoxin preparations are not currently available in the United States. In addition, the recent discovery that lethal toxin acts as a zinc metalloprotease inside target cells and the identification of potential target substrates may provide new insights for use in designing drugs that directly inhibit the toxicity of lethal factor in vivo.[14,17,18]

FUTURE CHALLENGES

Anthrax holds an important place in the development of modern medicine and has long been intertwined with human history. Anthrax is believed to have been one of the Egyptian plagues at the time of Moses, and cases were clearly recorded by the ancient Romans.[124] The anthrax bacillus was the model first used in the development of Koch's postulates and is considered the first "germ" proved to cause human disease.[125] Pasteur later generated a capsule-null anthrax strain that was the first vaccine made from live attenuated bacteria for use in humans.[126] At the birth of cellular immunology, Metchnikoff used the anthrax bacillus to examine the ability of his newly discovered macrophages to kill microbes.[127] Today, investigators are using *B. anthracis* and its toxins in an attempt to understand early events in the infectious process and the molecular basis of inflammation.[3,15,19]

Unfortunately, new issues have arisen beyond those related to scientific inquiry. No casualty-producing terrorist use of anthrax has occurred, and the Federal Bureau of Investigation has stated that it has "no intelligence that state sponsors of terrorism, international terrorist groups, or domestic terrorist groups are currently planning to use these deadly weapons in the United States."[128] However, the incidence of hoaxes has greatly increased with recent publicity about anthrax, providing a challenge to law enforcement.[129] Recent revelations regarding the development of anthrax weapons by the former Soviet Union and by Iraq, and of attempts to develop such weapons by the Aum Shinrikyo cult in Japan, make the potential use of *B. anthracis* in biologic terrorism a legitimate concern.[4,129] New strains resistant to antibiotics or containing additional virulence factors could be misused with the intent of confounding treatment or prophylaxis.[114,130] Whether our medical system would be able to provide appropriate prophylaxis and therapy in the event of a large-scale exposure to pathogenic endospores remains uncertain, even doubtful. It has now become relevant for physicians to refamiliarize themselves with clinical anthrax.

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