Chapter 6

ANTHRAX

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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.[1–5] Humans usually become infected by contact with infected animals or contaminated animal products, most commonly via the cutaneous route and only rarely via the respiratory or gastrointestinal routes.[6,7] 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. Virgil described anthrax in domestic and wild animals in his *Georgics*, and anthrax was an economically important agricultural disease during the 16th through 18th centuries in Europe.[8,9]

Anthrax, which is intimately associated with the origins of microbiology and immunology, was the first disease for which a microbial origin was definitively established. Robert Koch established the microbial origin for anthrax in 1876.[10,11] Anthrax also was the first disease for which an effective live bacterial vaccine was developed; Louis Pasteur developed that vaccine in 1881.[12] Additionally, anthrax represents the first described occupational respiratory infectious disease. During the latter half of the 19th century, inhalational anthrax,[13] a previously unrecognized form, occurred among wool-sorters in England as a result of the generation of infectious aerosols of anthrax spores under industrial conditions from the processing of contaminated goat hair and alpaca wool.[14]

The military has long been concerned about *B anthracis* as a potential biological weapon because anthrax spores are infectious by the aerosol route, and a high mortality rate is associated with untreated inhalational anthrax. In 1979 the largest inhalational anthrax epidemic of the 20th century occurred in Sverdlovsk, Russia. *B anthracis* spores were accidentally released from a military research facility located upwind from where the cases occurred. According to the accounts provided by two Soviet physicians, 96 human anthrax cases were reported, of which 79 were gastrointestinal and 17 cutaneous. The 79 gastrointestinal cases resulted in 64 deaths.[15] Although the initial report of this event attributed the infections to a gastrointestinal source, later evidence indicated that an aerosol release of weaponized anthrax spores from a military production facility had occurred, and thus, inhalational anthrax was the predominant cause of these civilian casualties. Retrospective analysis using administrative name lists of compensated families, household interviews, grave markers, pathologists' notes, various hospital lists, and clinical case histories of five survivors yielded evidence of 77 anthrax cases, with 66 deaths and 11 survivors.[15] Cases were also reported in animals located more than 50 km from the site.[16,17] Polymerase chain reaction examination of tissue samples collected from 11 of the victims demonstrated that virulent *B anthracis* DNA was present in all these patients, and at least five different strains of virulent *B anthracis* were detected based on variable number tandem repeat analysis.[18]

The retrospective data associated with the Sverdlovsk incident as well as studies performed for the Department of Homeland Security have been used by several computer modeling efforts to better understand the human infectious dose.[19,20] Under the direction of the Department of Health and Human Services, the Office of the Assistant Secretary of Preparedness and Response, Public Health Emergency Medical Countermeasures Enterprise, and Biomedical Advanced Research and Development, these agencies have developed a variety of computer dissemination models for a wide variety of potential scenarios.

Although the Sverdlovsk incident is not well known among US civilians, most people are familiar with the 2001 bioterrorist attack in the United States in letters containing dried *B anthracis* spores. The spore powder, which was sealed in letters addressed to members of Congress and the press, was mailed through the US Postal Service.[21–24] According to the Centers for Disease Control and Prevention, 22 people contracted anthrax from the letters.[21,25–29] Of the 11 individuals who developed inhalational anthrax, five died and six survived after intensive antimicrobial therapy. Eleven other people contracted cutaneous anthrax; all survived after treatment. Thousands of other persons received prophylaxis with antibiotics and, in some cases, postexposure vaccination.[30–33]

Considerable research has been devoted to biodefense research and modeling since this event.[34–45] It has been estimated that the 2001 anthrax attacks cost the United States more than $1 billion in medical planning, response, and remediation costs.[46–49] Additionally, this incident profoundly affected the law enforcement, scientific, and medical communities within the United States and throughout the world. Although the source of these letters has never been definitively identified, the impact on biodefense research establishments has been a transformational event for researchers and institutes.

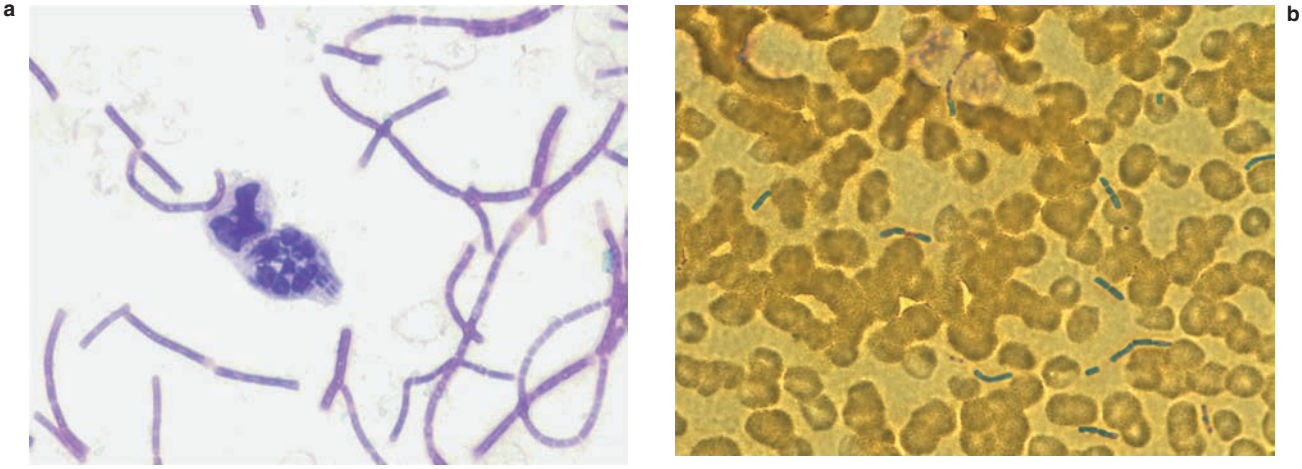

Figure 6-1. (**a**) Gram stain of a blood smear from an infected guinea pig demonstrating intracellular bacilli chains within a polymorphonuclear leukocyte. (**b**) Gram stain of peripheral blood smear from a nonhuman primate infected with *Bacillus anthracis*, Ames strain.
Photographs: (a) Courtesy of Susan Welkos, PhD, Division of Bacteriology, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland. (b) Courtesy of John Ezzell, PhD, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.

THE ORGANISM

B anthracis is a large, gram-positive, spore-forming, nonmotile bacillus (1–1.5 μm × 3–10 μm) that is closely related to *Bacillus cereus* and *Bacillus thuringiensis*. 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. The organism forms a prominent capsule both in vitro in the presence of bicarbonate in the culture media and elevated levels of carbon dioxide in the bacterial plate incubator and in tissue in vivo. In tissue, the encapsulated bacteria occur singly or in chains of two or three bacilli (Figure 6-1). The organism does not form spores in living tissue; sporulation occurs only after the infected carcass tissues are exposed to oxygen. The spores, which cause no swelling of the bacilli, are oval and they occur centrally or paracentrally (Figure 6-2). *B anthracis* spores are composed of dozens of spore coat proteins that—in part—protect the genomic material housed in the core.[50,51] The spores are surrounded by a loose fitting membrane referred to as the exosporium. The exosporium has been shown to impact how the spore interacts with certain types of mammalian cells.[52,53] The spores, which are resistant to environmental stressors, may survive for decades in certain soil conditions. Bacterial identification is confirmed by demonstration of the protective antigen (PA) toxin component, lysis by a specific bacteriophage, detection of capsule by fluorescent antibody, and virulence for mice and guinea pigs.[54,55] Additional confirmatory tests to identify toxin and capsule genes by polymerase chain reaction, developed as research tools, have been

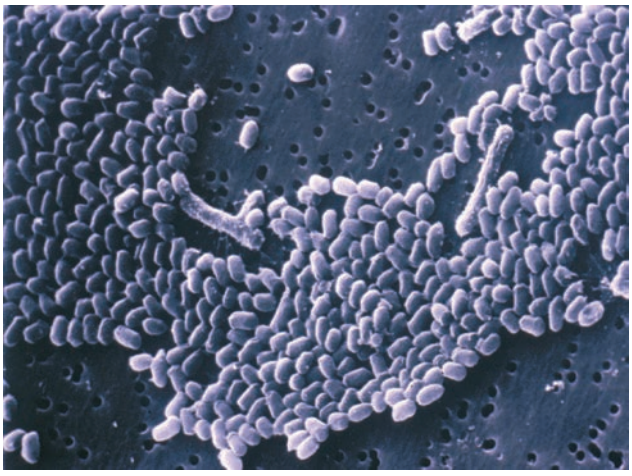

Figure 6-2. Scanning electron micrograph of a preparation of *Bacillus anthracis* spores. Two elongated bacilli are also presented among the oval-shaped spores. Original magnification × 2,620.
Photograph: Courtesy of John Ezzell, PhD, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.

incorporated into the Laboratory Response Network established by the Centers for Disease Control and Prevention.[56–59]

The diagnosis of anthrax has been complicated by the identification of strains of *B cereus*, which produce anthrax-like disease. Because *B cereus* is hemolytic and resistant to the anthrax-specific gamma bacteriophage, such isolates would not typically be tested for the presence of genes encoding anthrax toxin, especially because *B cereus* is often regarded as an environmental contaminant.[60] Continued reports of bacterial strains harboring anthrax toxin genes have demonstrated not only the importance of appropriate detection strategies, but also the possibility of emerging risks associated with the possible transfer of *B anthracis* characteristics to other organisms.[61,62]

EPIDEMIOLOGY

B anthracis, an organism that exists in the soil as a spore, occurs worldwide. Whether its persistence in the soil results from significant multiplication of the organism, or from cycles of bacterial amplification in infected animals whose carcasses then contaminate the soil, remains unsettled.[63–67] The form of the organism in infected animals is the bacillus.

Domestic or wild animals become infected when they ingest spores while grazing on contaminated land or eating contaminated feed. Pasteur originally reported that environmental conditions such as drought, which may promote trauma in the oral cavity on grazing, may increase the chances of acquiring anthrax.[68] Spread from animal to animal by mechanical means—by biting flies and from one environmental site to another by nonbiting flies and by vultures—has been suggested to occur.[64,69]

Anthrax in humans is associated with agricultural, horticultural, or industrial exposure to infected animals or contaminated animal products. In less developed countries, primarily Africa, Asia, and the Middle East, disease occurs from contact with infected domesticated animals or contaminated animal products. Contact may include handling contaminated carcasses, hides, wool, hair, and bones or ingesting contaminated meat. Cases associated with industrial exposure—rarely seen—occur in workers processing contaminated hair, wool, hides, and bones. Direct contact with contaminated material leads to cutaneous disease, and ingestion of infected meat leads to oropharyngeal or gastrointestinal forms of anthrax. It has been well documented that intravenous drug users can become infected with *B anthracis*, resulting in a septicemic form of anthrax.[70–78] 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, leads to inhalational anthrax. Military research facilities have played a major role in studying and defining anthrax, as well as many other zoonotic diseases in wild and domestic animals and the subsequent infections in humans.[79]

Unreliable reporting makes it difficult to estimate with accuracy the true incidence of human anthrax. It was estimated in 1958 that between 20,000 and 100,000 cases occurred annually worldwide.[80] In more recent years, anthrax in animals has been reported in 82 countries, and human cases continue to be reported from Africa, Asia, Europe, and the Americas.[81–85] In the 1996–1997 global anthrax report, a general decrease appeared in anthrax cases worldwide; however, anthrax remains underdiagnosed and underreported.[86]

In the United States the annual incidence of human anthrax has steadily declined from about 127 cases in the early part of the 20th century to about 1 per year for the past 10 years.[87] The vast majority of these cases have been cutaneous. Under natural conditions, inhalational anthrax is rare; before the anthrax bioterrorism event in 2001, only 18 cases had been reported in the United States in the 20th century.[88,89] In the early part of the 20th century, inhalational anthrax cases were reported in rural villagers in Russia who worked with contaminated sheep wool inside their homes.[90] However, in recent years a significant decrease occurred in anthrax cases in domestic animals in east Russia. Five inhalational anthrax cases occurred in woolen mill workers in New Hampshire in the 1950s.[91] During economic hardship and disruption of veterinary and human public health practices (eg, during wartime), large anthrax epidemics have occurred. The largest reported human anthrax epidemic occurred in Zimbabwe from 1978 through 1980, with an estimated 10,000 cases.[92]

Essentially all cases were cutaneous, including rare gastrointestinal disease cases and eight inhalational anthrax cases, although no autopsy confirmation was reported.[93]

PATHOGENESIS

B anthracis produces two protein exotoxins, known as the lethal toxin (LT) and the edema toxin (ET); an antiphagocytic capsule; and other known and putative virulence factors.[94] The role of the capsule in pathogenesis was demonstrated in the early 1900s, when anthrax strains lacking a capsule were shown

to be attenuated.[95] In more recent years, the genes encoding synthesis of the capsule were identified on the 96-kilobase plasmid known as pXO2. 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.[96] The capsule is composed of a polymer of D-glutamic acid, which confers resistance to phagocytosis and may contribute to the resistance of anthrax to lysis by serum cationic proteins.[97–102] Capsule production is necessary for dissemination to the spleen in a murine inhalational anthrax model.[103] The capsule has also been the focus of several efforts to develop new generation anthrax vaccines.[104–106] Evidence indicates that the capsule may enhance the protection afforded by PA-based vaccines against anthrax if opsonizing antibodies are produced.[106]

Koch first suggested the importance of toxins in his initial studies on anthrax. In 1954 Smith and Keppie[107] 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 researchers in the ensuing years.[108,109] Advances in molecular biology have produced a more complete understanding of the biochemical mechanisms of action of the toxins, and they have begun to provide a more definitive picture of their role in the pathogenesis of the disease.

Two protein exotoxins, known as the LT and the ET, are encoded on a 182-kb plasmid (pXO1), distinct from that coding for the capsule. In an environment of increased bicarbonate in the growth media, atmospheric carbon dioxide within the plate incubation chamber, and increased temperature, such as is found in the infected host, transcription of the genes encoding these and other virulence-associated gene products is enhanced.[94,110–113] A complex regulatory cascade controlled in large part by the *atxA* and *acpA* genes encoded on the toxin plasmid pXO1 and pXO2, respectively, directs the production of virulence factors in response to these environmental signals.[114,115] The anthrax toxins, like many bacterial and plant toxins, possess two components: (1) a cell binding, pore-forming, or B, domain; and (2) an active, or A, domain that has the toxic and—usually—the enzymatic activity (Figure 6-3). The B and A anthrax toxin components, which are synthesized from different genes, are secreted as noncovalently linked proteins. The anthrax toxins are unusual because both toxins share the B protein, PA. Thus, the LT is composed of the PA63 (MW [molecular weight] 63,000 after cleavage from a MW 83,000 protein) heptamer or octamer combined with a second protein, which is known as lethal factor (LF [MW 90,000]), and the ET is composed of PA complexed with the edema factor (EF [MW 89,000]). Each of these 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 pXO1[96,116] or the PA gene alone[117] attenuates the organism. Crude toxin preparations have been shown to impair neutrophil chemotaxis[118,119] and phagocytosis.[97]

The ET, which causes edema when injected into the skin of experimental animals, is likely responsible for the marked edema often present at bacterial replication sites.[120,121] This toxin is a calmodulin-dependent adenylate cyclase that impairs phagocytosis and priming for the respiratory burst in neutrophils; it also inhibits the production of interleukin-6 and tumor necrosis factor by monocytes, which may further weaken host resistance.[122–124] ET also impairs dendritic cell function and appears to act with LT to suppress the innate immune response.[125]

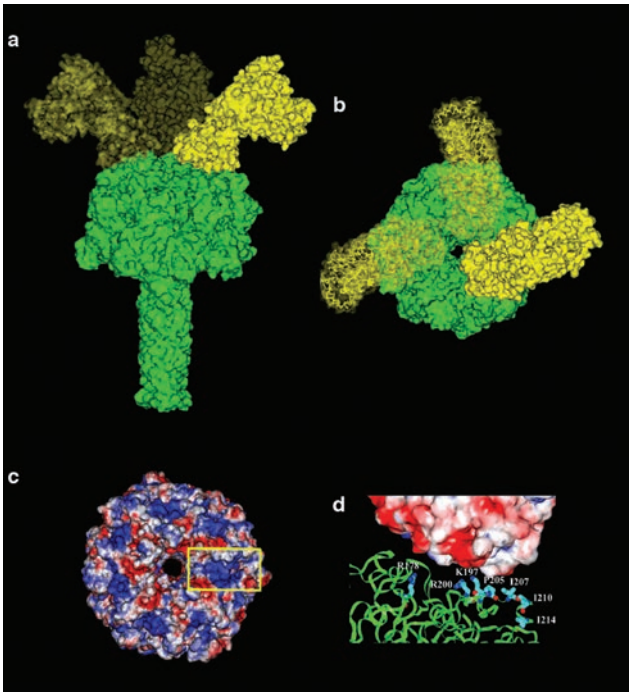

Figure 6-3. Composition of anthrax lethal protein toxin. Molecular models of the protective antigen $(PA)_{63}$ heptamer and the PA_{63} heptamer-lethal factor (LF) complex. (**a, b**) Side and top views of PA_{63} heptamer (*green*) bound to three LF molecules (*yellow*). (**c, d**) The surface renderings are colored according to the negative (*red*) and positive (*blue*) electrostatic surface potential. (**c**) Top view of the PA_{63} heptamer. The *yellow box* highlights the protomer-protomer interface and where LF binds to heptameric PA. (**d**) A hypothetical PA_{63} heptamer–LF interface.
Photographs: Courtesy of Kelly Halverson, PhD, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.

The LT is a zinc metalloprotease that is lethal for experimental animals[120,121,126] and is directly cytolytic for rodent macrophages, causing release of the potentially toxic cytokines interleukin-1 and tumor necrosis factor.[127] In in vitro models, LT cleaves members of the mitogen-activated protein kinase (MAPK) kinase family, which are an integral part of a phosphorelay system that links surface receptors to transcription of specific genes within the nucleus. Thus, LT interferes with the MAPK signaling pathways necessary for many normal cell functions.[128] In macrophage and dendritic cell models, LT leads to inhibition of proinflammatory cytokines, downregulation of costimulatory molecules, and ineffective T-cell priming.[128–131] In vitro it also appears to promote apoptosis of endothelial cells lining the vascular system, leading to speculation that LT-induced barrier dysfunction leads to the vascular permeability changes accompanying systemic anthrax infection.[132] Effects on hormone receptors, including glucocorticoids, have also been reported. Although much of the information regarding LT activity has been obtained from animal-derived cell culture models, Fang et al reported that—in vitro—LT inhibits MAPK kinase dependent interleukin-2 production and proliferative responses in human CD4+ T cells.[133] Studies using tissue-specific CMG2 knockout mice strongly indicate that LTs/ETs target myeloid-derived cells to promote bacterial survival early in infection.[134] In addition, the data suggest that elevated levels of toxin specifically target host organs and are responsible for the significant morbidity and mortality caused by anthrax infection.[135]

Studies in cell culture models have provided a clearer understanding of the molecular interactions of the toxin proteins.[128] PA first binds, most likely by a domain at its carboxy-terminus, to a specific cell receptor.[136–138] Two proteins have been proposed as the PA receptor: (1) Tumor endothelial marker 8 TEM8, (ANTX1); and (2) capillary morphogenesis protein, CMG2 (ANTX2).[139–141] Both receptors have a von Willibrand factor type A domain that appears to interact with PA. Once bound, PA is cleaved by a furin-like protease, resulting in retention of a 63-kilodalton fragment of PA on the cell surface.[142,143] This cleavage promotes formation of PA heptamers and creates a binding site on PA to which up to three molecules of the LF and the EF can bind with high affinity.[129] Heptamerization[144] and octamerization[141–144] stimulates endocytosis of PA (or PA EF or PA LF complexes), which are then delivered into early endosomes. The mildly acidic pH of the endosome is hypothesized to trigger membrane insertion of the heptameric PA into intraluminal vesicles.[145] EF and LF are translocated into the lumen of the vesicle and are thereby protected from lysosomal proteases.[145] The toxins are then translocated via endosomal carrier vesicles to the cell cytosol, where they express their toxic activity.[145] In addition, studies have also suggested that the formation of octamers provides stability to these toxin products and permits active LT to travel freely in the circulatory system.[146]

The processes leading to toxin activity in the infected animal may be more complicated because the toxin proteins appear to exist in the serum as a complex of PA and EF/LF.[147] The proteolytic activation of PA necessary to form LT or ET may occur in interstitial fluid or serum rather than on the cell surface.[147] The LT or ET may then bind to target cells and be internalized. This theory was bolstered by Panchal et al who demonstrated that purified LF complexed with the PA heptamer cleaved both a synthetic peptide substrate and endogenous MAPK kinase substrates and killed susceptible macrophage cells.[148] In addition, complexes of the heptameric PA-LF found in the plasma of infected animals showed functional activity.[148] Terminally, toxin is present in very high concentrations in the blood, which probably accounts for the sudden death observations in infected experimental animals.

Although these toxins were once thought to be exclusively found in *B anthracis*, recent cases of inhalational disease have been identified that possess the hallmarks of anthrax disease; however, the bacteria recovered were not *B anthracis* but did possess anthrax toxin genes.[149–152] Studies have identified isolates of *B cereus* that carried a plasmid homologous to the anthrax toxin plasmid pXO1. The polyglutamate capsule was not produced by this *B cereus* isolate. However, gene sequences encoding a polysaccharide capsule were present on a smaller plasmid.[149] Capsule-producing strains of *B cereus* have caused severe pneumonia.[150] Consequently, a possibility of false positives exists in diagnostic tests that rely on toxin-based identification of genes or gene products. Subsequent investigations of these strains determined that the virulence of these strains in mice, guinea pigs, and rabbits was significantly attenuated when compared to fully virulent *B anthracis*.[153,154] It was also shown that vaccines that are effective against fully virulent *B anthracis* can protect mice and guinea pigs from infection with the anthrax-like *B cereus* strain.[154]

Infection begins when the spores are introduced through the skin or mucosa. Spores are then ingested at the local site by macrophages. Phagocytosed spores can have multiple fates depending on the stage of infection and the spore burden of individual phagocytes.[155,156] Within the lungs, spores are translocated by pulmonary macrophages and dendritic cells. Phagocytes have a dual role; they can transport spores to the lymphatic system[157–160] but also are bactericidal toward

germinating spores under certain conditions.[158,161–163] Another hypothesis has been proposed that may explain the toxins' effects early in the infectious process. Banks, Ward, and Bradley[164] have hypothesized that intoxication may occur after spores have been engulfed by phagocytic cells. The anthrax toxin receptors have been located on the inside of the phagolysosome, and the germinating spore may secrete toxins that interact with these receptors within the phagolysosome. The effector molecules (EF and/or LF) can then be translocated into the cytoplasm.[155,164]

Once a spore becomes vegetative, it can produce a robust capsule and large amounts of toxins. At these sites, the bacteria proliferate and produce the ETs and LTs that impair host leukocyte function and lead to the distinctive pathological findings: edema, hemorrhage, tissue necrosis, and a relative lack of leukocytes. Once the vegetative cells emerge from the phagolysosome, they replicate within the cell and finally exit through the host cell plasma membrane.[160] In inhalational anthrax, the spores are ingested by alveolar phagocytes, which transport them to the regional tracheobronchial lymph nodes, where germination occurs.[165]

Anthrolysin O (ALO) and phospholipases may also play critical roles as virulence factors for *B anthracis*[166] and mediate the toxicity of *B anthracis* to lung epithelial cells under microaerobic conditions.[167] ALO has been found to cause lysis of human phagocytes and epithelial cells. The mechanism of action appears to be from ALO pore-forming alterations of the cellular membrane, resulting in acute primary membrane permeabilization followed by a burst of reactive radicals released from the mitochondria.

The evidence reported from animal studies overwhelmingly suggests that the alveolar spaces are not permissive for significant levels of spore germination. Rather, spores begin to germinate once phagocytosed during translocation to and upon deposition within lymph nodes.[165,168–171] However, several studies have suggested that small amounts of germination may occur within the alveolar spaces.[171,172] Additionally, the nasal-associated lymphoid tissue has been explored as another area from which infection may be initiated.[171,173,174] These data, largely collected through in-vivo imaging technologies, suggest that other scenarios may lead to spore germination after inhalation.[159] Once in the tracheobronchial lymph nodes, the local production of toxins by extracellular bacilli generates the characteristic pathology picture: massive hemorrhagic, edematous, and necrotizing lymphadenitis; and mediastinitis (the latter is almost pathognomonic of this disease).[175]

These findings in human disease have been replicated in various animal disease models.[176,177] The bacilli can then spread to the blood, leading to septicemia with seeding of other organs and frequently causing hemorrhagic meningitis. Death is most likely the result of systemic inflammatory response syndrome triggered by the release of endogenous cellular contents from damaged or dying cells, termed damage-associated molecular patterns and in combination with exogenous microbial exposure or pathogen-associated molecular patterns,[178] resulting in respiratory failure associated with pulmonary edema, direct cardiac tissue damage, overwhelming bacteremia, accompanied frequently with meningitis.

CLINICAL DISEASE

The military seeks to defend against anthrax used as an inhalational biological weapon. However, other anthrax forms are 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 anthrax cases are cutaneous.[179–181] 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 serosanguineous fluid with many organisms and a paucity of leukocytes. Histopathology findings consist of varying degrees of ulceration, vasculitis, perivascular inflammation, coagulative necrosis, hemorrhage, and edema.[182] The vesicle—which may be 1 to 2 cm in diameter—ruptures, leaving a necrotic ulcer (Figure 6-4). Satellite vesicles may also be present. The lesion is usually painless, and varying degrees of edema may be present around it.[183] The edema may occasionally be massive, encompassing the entire face or limb, which is described as "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 2 to 3 weeks the eschar separates, often leaving a scar and sometimes requiring surgical reconstruction.[184,185] Debridement has been shown to improve survival rates in a mouse model of subcutaneous anthrax[159]; however, no clinical studies have been conducted to validate this procedure in human clinical disease. Septicemia is rare, and with treatment, mortality should be less than 1%.[184,186–188] In addition, no age-related risk factor appears to be associated with cutaneous human anthrax.[189]

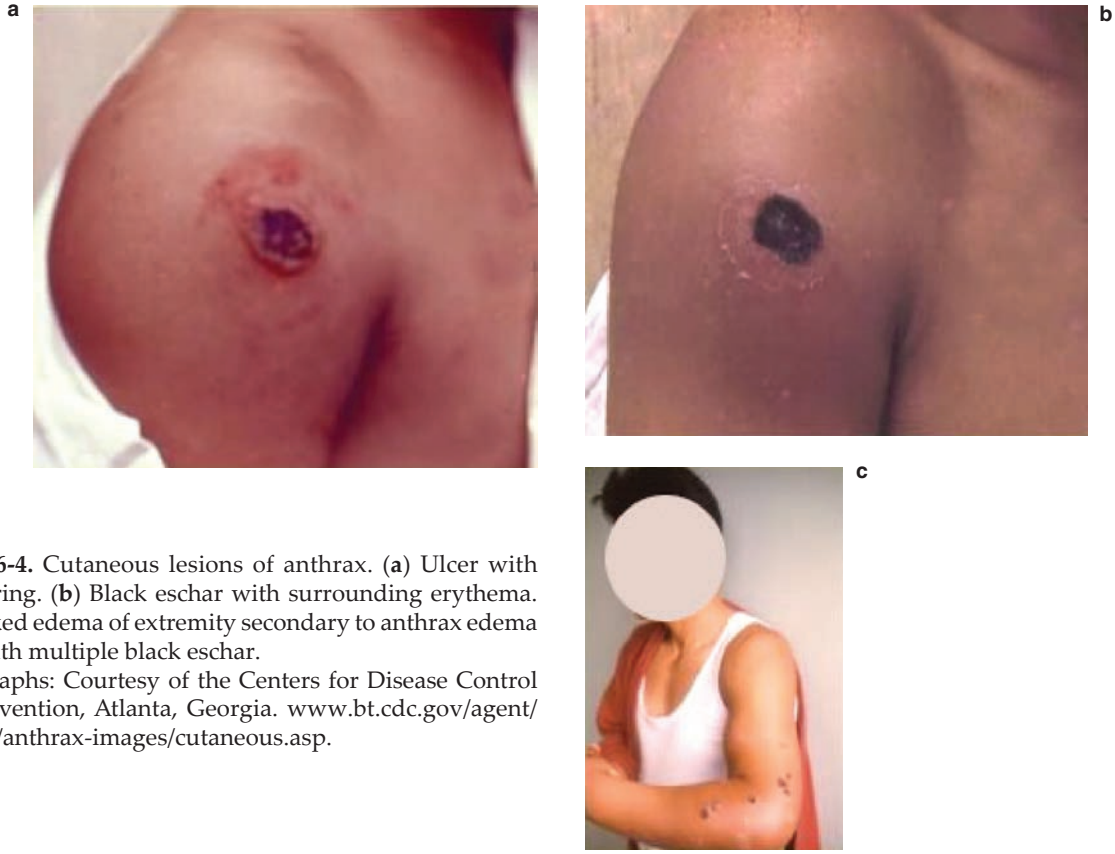

Figure 6-4. Cutaneous lesions of anthrax. (**a**) Ulcer with vesicle ring. (**b**) Black eschar with surrounding erythema. (**c**) Marked edema of extremity secondary to anthrax edema toxin with multiple black eschar.
Photographs: Courtesy of the Centers for Disease Control and Prevention, Atlanta, Georgia. www.bt.cdc.gov/agent/anthrax/anthrax-images/cutaneous.asp.

Of recent interest has been the identification of anthrax cases among intravenous drug users in western Europe.[70–75] In 2000 a case of cutaneous anthrax was identified in a Norwegian patient who participated in subdermal drug injection, commonly known as "skin popper."[76] The first reported case of intravenous drug user-associated anthrax was in Scotland with subsequent 47 confirmed cases and 13 fatalities. These numbers increased to a total of 119 cases from December 2009 to December 2010.[74] This disease is thought to be initiated by direct injection of spore-contaminated heroin, which led to clinical presentations ranging from subcutaneous disease to septicemic anthrax.[70–78,190–192]

Inhalational Anthrax

Inhalational anthrax begins after an incubation period of 1 to 6 days with nonspecific symptoms of malaise, fatigue, myalgia, and fever.[193–195] A nonproductive cough and mild chest discomfort may also occur. These symptoms usually persist for 2 or 3 days, and in some cases there may be a short period of improvement. Then a sudden onset of increasing respiratory distress with dyspnea, stridor, cyanosis, increased chest pain, and diaphoresis occurs. Associated edema of the chest and neck may also be present. Chest radiograph examination usually shows the characteristic widening of the mediastinum from necrosis and hemorrhage of the lymph nodes and surrounding tissues, often with associated pleural effusions (Figure 6-5). In the 2001 bioterrorist event, the pleural effusions were initially small but rapidly progressed and persisted despite effective antibiotic therapy.[195,196] The effusions were predominantly serosanguineous, and immunohistochemistry revealed the presence of *B anthracis* cell wall and capsule antigens. Effusion fluid from deceased patients who had received fewer than 55 hours of antibiotic therapy revealed bacilli.[197]

Polymerase chain reaction analysis of the pleural fluid was also positive for *B anthracis* DNA.[198] Pneumonia has not been a consistent finding but can occur in some patients and may be attributed to vascular permeability, intra-alveolar edema, and hyaline membrane formation.[197] Although inhalational anthrax cases have been rare in this century, except for the 11 cases arising from the anthrax letters in 2001, several cases have occurred in patients with underlying pulmonary disease, suggesting that this condition may increase susceptibility to the disease.[68] Meningitis is present in up to 50% of cases, and some patients may present with seizures. The onset of respiratory distress is fol-

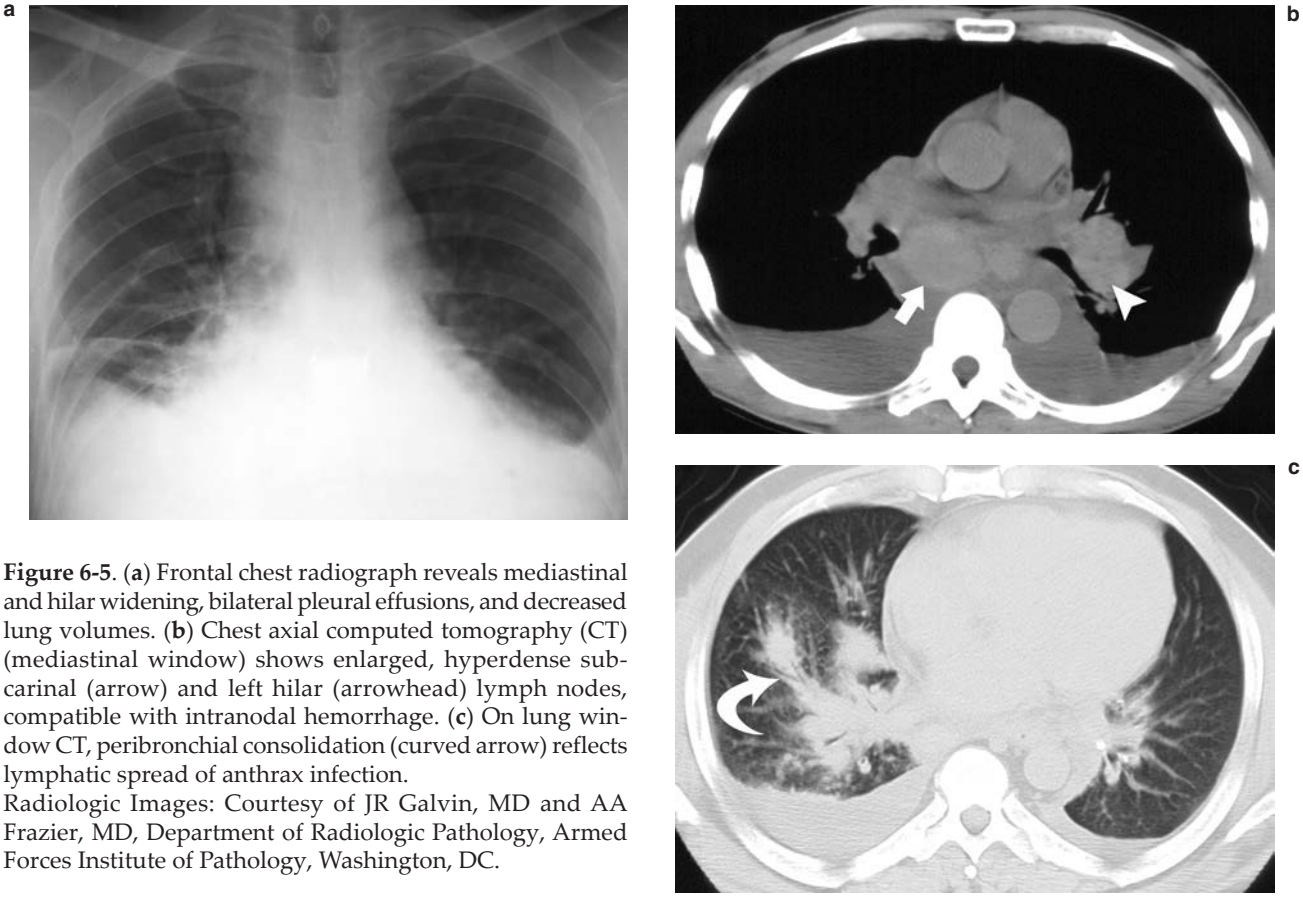

Figure 6-5. (**a**) Frontal chest radiograph reveals mediastinal and hilar widening, bilateral pleural effusions, and decreased lung volumes. (**b**) Chest axial computed tomography (CT) (mediastinal window) shows enlarged, hyperdense subcarinal (arrow) and left hilar (arrowhead) lymph nodes, compatible with intranodal hemorrhage. (**c**) On lung window CT, peribronchial consolidation (curved arrow) reflects lymphatic spread of anthrax infection.
Radiologic Images: Courtesy of JR Galvin, MD and AA Frazier, MD, Department of Radiologic Pathology, Armed Forces Institute of Pathology, Washington, DC.

lowed by the rapid onset of shock and death within 24 to 36 hours. Mortality had been essentially 100% in the absence of appropriate treatment; however, during 2001 the mortality rate was 45%.[195,196]

An inhalational pulmonary disease thought initially to be anthrax has been identified to be caused by *B cereus* strains.[152,199] These cases were found in metal welders, and susceptibility of these patients to this unusual pathogen may be related to inhalation of heavy metals during welding. Heavy metal exposure produces immunosuppression and an increased susceptibility to infection.

Meningitis

Meningitis may occur after bacteremia as a complication of any of the disease's clinical forms.[190–192] Meningitis may also occur—rarely—without a clinically apparent primary focus, and it is often hemorrhagic, which is important diagnostically, and almost always fatal (Figure 6-6). Studies have suggested that LF, EF, and protease InhA inhibit neutrophil signaling pathways in brain endothelium, thus promoting anthrax meningitis.[193–195]

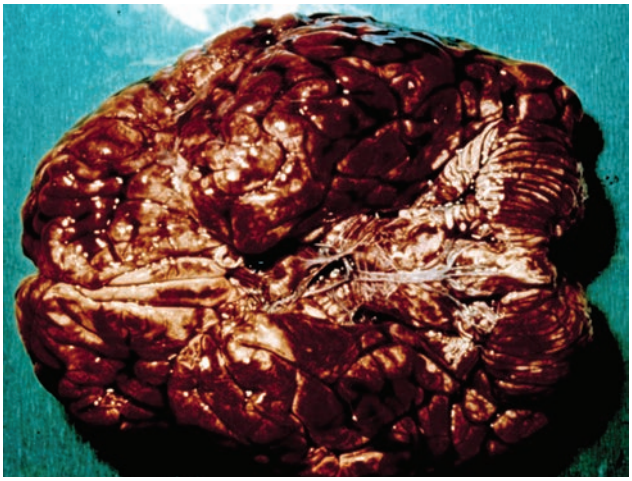

Figure 6-6. Meningitis with subarachnoid hemorrhage in a man from Thailand who died 5 days after eating undercooked carabao (water buffalo).
Reproduced from: Binford CH, Connor DH, eds. *Pathology of Tropical and Extraordinary Diseases.* Vol 1. Washington, DC: Armed Forces Institute of Pathology; 1976: 121. AFIP Negative 75-12374-3.

Oropharyngeal and Gastrointestinal Anthrax

Oropharyngeal and gastrointestinal anthrax result from ingesting infected meat that has not been sufficiently cooked or by ingesting anthrax spores either directly or from swallowing contaminated respiratory secretions.[178,200,201] 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 resulting from 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; in most cases severe abdominal pain follows. The presenting sign may be an acute abdomen, which may be associated with hematemesis, massive ascites, and bloody diarrhea. Mortality in both forms may be as high as 50%, especially in the gastrointestinal form.

DIAGNOSIS

The most critical aspect in making an anthrax diagnosis is a high index of suspicion associated with a compatible history of exposure. Cutaneous anthrax should be considered after a painless pruritic papule, vesicle, or ulcer develops—often with surrounding edema—and then becomes a black eschar. With extensive or massive edema, such a lesion is almost pathognomonic. Gram stain or culture of the lesion usually confirms the diagnosis. Bacterial culture tests include colony morphology on sheep blood agar plates incubated at 35°C to 37°C for 15 to 24 hours. *B anthracis* colonies are 2 to 5 mm in diameter, flat or slightly convex, irregularly round with possible comma-shaped ("Medusa-head") projections with a ground-glass appearance (Figure 6-7). The colonies tend to have tenacious consistency when moved with a bacterial loop and are not β-hemolytic. The bacteria appear as gram-positive, 1 to 8 μm long and 1 to 1.5 μm wide bacilli. India ink staining reveals capsulated bacteria. A motility test should be performed either by wet mount or motility media; *B anthracis* is nonmotile. Gamma bacteriophage lysis and direct fluorescent antibody tests are performed at Level D laboratories as confirmatory tests (Figures 6-7 and 6-8). Commercial polymerase chain reaction kits specific for the *B anthracis* pXO1 and pXO2 plasmids are also available to assist in identifying this organism. 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 difficult, but the disease should be suspected with a history of exposure to a *B anthracis*–containing aerosol. The early

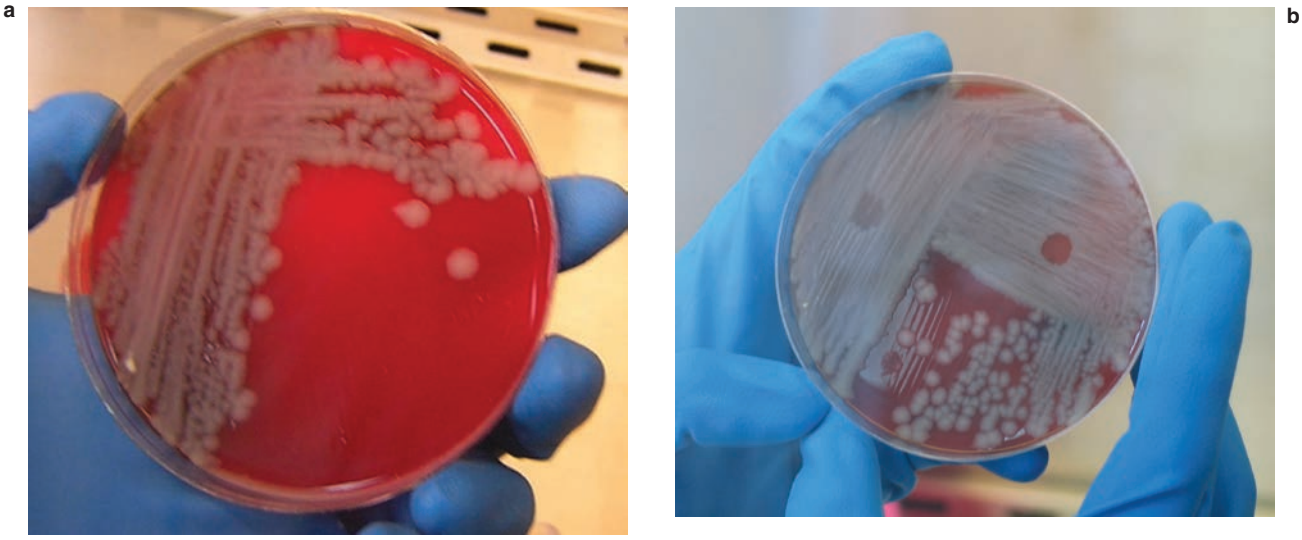

Figure 6-7. (**a**) Isolated colonies of *Bacillus anthracis* on sheep blood agar plate. (**b**) Detection of *B anthracis* using specific gamma-phage mediated cell-lysis.
Photographs: Courtesy of Bret K Purcell, PhD, MD, Division of Bacteriology, US Army Medical Research Institute of Infectious Diseases and the Defense Threat Reduction Agency/Threat Agent Detection and Response Program, National Center for Disease Control, Tbilisi, Georgia, 2005.

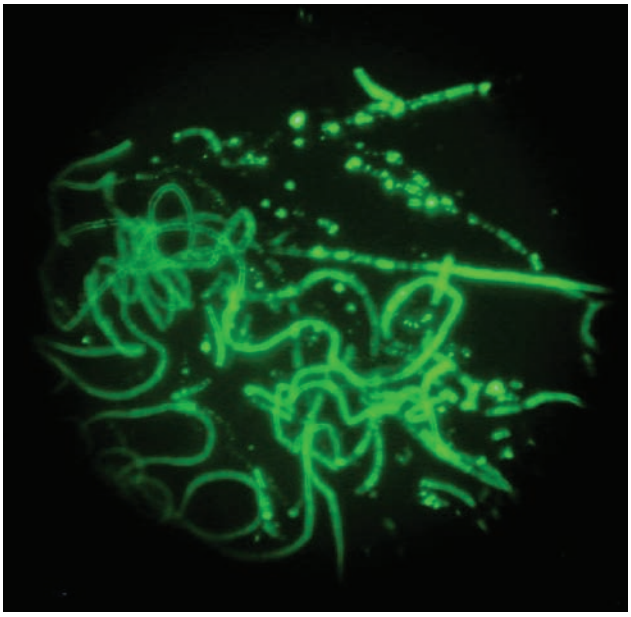

Figure 6-8. Direct fluorescent antibody stain of *Bacillus anthracis* capsule.
Photograph: Courtesy of David Heath, PhD, Division of Bacteriology, US Army Medical Research Institute of Infectious Diseases and the Defense Threat Reduction Agency/Threat Agent Detection and Response Program, National Center for Disease Control, Tbilisi, Georgia, 2005.

symptoms are nonspecific[194,202–204] and include fever, chills, dyspnea, cough, headache, vomiting, weakness, myalgias, abdominal pain, and chest or pleuritic pain. This stage of the disease may last from hours to a few days. However, the development of respiratory distress in association with radiographic evidence of a widened mediastinum resulting from hemorrhagic mediastinitis and the presence of hemorrhagic pleural effusion or hemorrhagic meningitis should suggest the diagnosis. Contrast-enhanced computer tomography images reveal diffuse hemorrhagic mediastinal and hilar adenopathy with edema, perihilar infiltrates, bronchial mucosal thickening, and hemorrhagic pleural, and pericardial effusions.[205] During the later stages of the disease patients develop sudden fever, dyspnea, diaphoresis, cyanosis, hypotension, shock, and death.[202] Blood culture should demonstrate growth in 6 to 24 hours if the patient has not received antibiotics before collection, and a Gram stain of peripheral blood smears often reveals large bacilli in later disease stages. Sputum examination is not helpful in making the diagnosis because pneumonia is usually not a feature of inhalational anthrax.

Gastrointestinal anthrax is difficult to diagnose because of its rarity and nonspecific symptoms including nausea, vomiting, anorexia, and fever. As the disease progresses, patients often develop acute, severe abdominal pain, hematemesis, and bloody diarrhea. Diagnosis is usually considered only with a history of ingesting contaminated meat in the setting of an outbreak. Microbiological cultures do not help confirm the diagnosis. The diagnosis of oropharyngeal anthrax can be made from the clinical and physical findings in a patient with the appropriate epidemiological history. Sore throat, dysphagia, hoarseness, cervical lymphadenopathy, and edema as well as fever are often presenting symptoms.[194,206,207]

Meningitis resulting from anthrax is clinically indistinguishable from meningitis attributable 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 useful in making a retrospective diagnosis. Antibody to PA or the capsule develops in 68% to 93%[208–211] of reported cutaneous anthrax cases and 67% to 94%[210,211] of reported oropharyngeal anthrax cases. 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[212] to help with the retrospective anthrax diagnosis. Western countries have limited experience with this test.[213] The Food and Drug Administration (FDA) has recently approved two tests: (1) the QuickELISA Anthrax-PA Kit (Immunetics, Boston, MA) for identification of PA toxin in blood from infected human casualties, and (2) the PCR Joint Biological Agent Identification and Diagnostic System (Idaho Technology Inc, Salt Lake City, UT) anthrax test for rapid identification of bacteria in blood and blood culture samples.[214]

TREATMENT

Cutaneous anthrax without toxicity or systemic symptoms may be treated with oral penicillin if the infection did not originate with a potential aerosol exposure. However, if an inhalational exposure is also suspected, ciprofloxacin or doxycycline is recommended as first-line therapy.[202,203,215] Effective therapy reduces edema and systemic symptoms but does not change the evolution of the skin lesion. Treatment should be continued for 7 to 10 days, unless inhalational exposure is suspected; then treatment should be continued for 60 days. However, recent studies of the 2001 bioterrorism event have identified problems associated with pro-

longed treatment, mass prophylaxis, and medication compliance.[216–221] Amoxicillin is recommended for patients who cannot take fluoroquinolones or tetracycline-class drugs; however, increasing evidence shows that *B anthracis* possesses β-lactamase genes that may reduce the efficacy of this treatment.[222–227] In addition, if a bioterrorism event occurs, the bacterial strains used may be naturally antibiotic resistant or genetically modified to confer resistance to one or more antibiotics.

Tetracycline, erythromycin, and chloramphenicol have also been used successfully[228] for treating rare cases caused by naturally occurring penicillin-resistant organisms. Additional antibiotics shown to be active in vitro include gentamicin, cefazolin, cephalothin, vancomycin, clindamycin, and imipenem.[229–231] These drugs should be effective in vivo, but no reported clinical experience exists. Experimental infections using the inhalational mouse model have demonstrated significant efficacy using these additional antibiotics.

Inhalational, oropharyngeal, and gastrointestinal anthrax should be treated with intravenous therapy using two or more antibiotics. The therapy should initially include a fluoroquinolone or doxycycline with one or more of the following antibiotics: clindamycin, rifampin, penicillin, ampicillin, vancomycin, amino-glycosides, chloramphenicol, imipenem, and/or clarithromycin.[202,215] Tactical Combat Casualty Care guidelines have been established for medical management of patients in chemical, biological, radiological, nuclear, or high-yield explosives environments.[232] The Centers for Disease Control and Prevention issued guidelines for the treatment and management of human anthrax disease.[205,206] New guidelines published in 2014 recommend linezolid over clindamycin—when appropriate—to prevent toxin formation and the use of adjunctive corticosteroids when indicated.[233–236] The World Health Organization has also issued guidelines for the surveillance and control of anthrax in humans and animals and can be accessed at the following website: http://www.who.int/csr/resources/publications/anthrax/WHO_EMC_ZDI_98_6/en/.

Patients often require intensive care unit support, including appropriate vasopressors, oxygen, and other supportive therapy, because of the disease's severity and rapid onset. Recommendations for treatment during pregnancy and for pediatric populations follow similar guidelines.[234–236] The development of severe bacterial sepsis has been well documented for anthrax in both human clinical disease and experimental animal models. The expression of LT and ET as well as other virulence factors such as ALO promote the development of systemic inflammatory response syndrome by both damage-associated molecular patterns and pathogen-associated molecular patterns.[178] This immunologic stimulation, if unregulated or limited, results in the formation of a cytokine cascade and eventual storm resulting in multiorgan system failure and rapid death of humans exposed to inhalational anthrax as well as other select agents. This immunologic over-response has prompted the evaluation of various augmentation therapies to mitigate these events. One such therapy that received FDA approval in 2012 is raxibacumab (GlaxoSmithKline, Brentford, Middlesex, United Kingdom), a human IgG1 monoclonal antibody directed against the PA antigen of *B anthracis*.[237,238] This product was the first monoclonal antibody approved for use in the treatment of severe inhalational anthrax under the FDA's Animal Efficacy Rule.[237–239] The study found that 64% of *Cynomolgus macaque* monkeys and 44% of rabbits with inhalational anthrax survived, whereas all placebo control animals died from both groups.[239] An additional study comparing antibiotics and raxibacumab against antibiotics demonstrated a 82% survival for combination therapy versus 65% for antibiotics only. When rabbits were treated with levofloxacin plus raxibacumab verses levofloxacin alone, the absolute difference in survival rates between the groups was not statistically significant; however, clinically there was only an 18% death rate in the levofloxacin plus raxibacumab group and a 35% death rate in the levofloxacin only group.[240,241]

PROPHYLAXIS

Prophylactic Treatment After Exposure

Experimental evidence[242] has demonstrated that treatment with antibiotics (including ciprofloxacin, doxycycline, and penicillin) beginning 1 day after exposure to a lethal aerosol challenge with anthrax spores can significantly protect against death. Combining antibiotics with active vaccination provides the optimal protection. Recent analysis has suggested postexposure vaccination may shorten the duration of antibiotic prophylaxis, providing the least expensive and most effective strategy to counter a bioterrorism event.[243–245]

Active Immunization

Emergent BioSolutions (Rockville, MD) produces the only licensed human vaccine against anthrax, anthrax vaccine adsorbed (BioThrax). 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 83-kDa PA, is adsorbed to 1.2 mg/mL of aluminum hydroxide in 0.85% sodium chloride. The final product also contains 100 μg/mL of formaldehyde and 25 μg/mL of benzethonium chloride as preservatives. Some vaccine lots contain small amounts of LF and lesser amounts of EF, as determined by antibody responses in vaccinated animals.[246,247] Low levels of antibody to LF and EF by Western blot have been reported in some vaccines, but these did not contribute significantly during toxin neutralization assays.[248] The vaccine is stored at 2°C to 8°C. 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 and laboratory workers should also be vaccinated. Vaccination is also indicated for protection against anthrax use in biological warfare.

Recommendations have been made for anthrax vaccine use in the United States.[249,250] The current guidelines recommend the anthrax vaccine adsorbed vaccine should be administered to prime the immune system to prevent infection as either a preexposure vaccine or after exposure to aerosolized *B anthracis* pores. For preexposure protection the Advisory Committee on Immunization Practices recommends intramuscular injections starting on day 0 followed by week 4, and every 6 months (6, 12, and 18 months) for a total of 5 doses as the initial vaccination series. Since no in vitro correlate of immunity exists for humans, annual boosters are recommended if the potential for exposure continues. For postexposure to anthrax, those persons who have been previously unvaccinated should receive the vaccine as a three dose, subcutaneous series (at 0, 2, and 4 weeks) in addition to the administration of a 60-day course of an appropriate antimicrobial therapeutic.

More than 2.6 million US military personnel have received the licensed anthrax vaccine adsorbed vaccine, and no unusual rates of serious adverse events have been noted.[251] Additional studies also support the safety of the anthrax vaccine.[252–260] The next generation vaccine, recombinant PA, may afford equivalent protection with a decrease in reactogenicity. A live attenuated, unencapsulated spore vaccine is used for humans in the former Soviet Union. The vaccine is given by scarification or subcutaneously. Its developers claim that it is reasonably well tolerated and shows some degree of protective efficacy against cutaneous anthrax in clinical field trials.[212] New attenuated vaccines developed in the United States are being evaluated for efficacy in inhalational anthrax animal models.[261] Recent studies have demonstrated a fourfold rise in anti-PA immunoglobulin G (IgG) titers of 85% and 100% in adults receiving two and three doses, respectively, of either subcutaneous or intramuscular AVA.[262–265]

One hundred percent of the vaccinees developed a rise in titer in response to the yearly booster dose. When tested by an enzyme-linked immunosorbent assay, the current serologic test of choice, more than 95% of vaccinees seroconvert after the initial three doses.[248]

A rough correlation exists between antibody titer to PA and protection of experimental animals from infection after vaccination with the human vaccine. However, the exact relationship between antibody to PA 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 PA.[246–248]

A recent study evaluating the response of mice to recombinant PA revealed significant variation of fine specificity of humoral response to the antigen even among genetically identical mice using the same immunogen and environment.[266,267] The authors demonstrated a heterogeneity of response to the PA antigen and identified specific epitopes that correlated to seroconversion and LeTx neutralization. Then they speculated that this observed stochastic variation in humoral immunity was likely a major contributing factor to the heterogeneity of vaccine response. Although these data suggest enhancing immunologic recognition of specific epitopes can improve vaccine protective response, the current anthrax vaccine adsorbed vaccine has demonstrated significant protection to nonhuman primates when exposed to inhalational challenge with large doses of anthrax spores.[268–274]

The protective efficacy of experimental PA-based vaccines produced from sterile culture filtrates of *B anthracis* was clearly demonstrated by various animal models and routes of challenge.[275] A placebo-controlled clinical trial was conducted with a vaccine similar to the currently licensed US vaccine.[276]

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.[277]

This vaccine 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 inhalational anthrax cases to determine whether the vaccine was effective. This same vaccine was previously shown to protect rhesus monkeys and other animal models against an aerosol exposure to anthrax spores.[277–282] No controlled clinical trials in humans of the efficacy of the currently licensed US vaccine have been conducted. This vaccine has been extensively tested in animals and has protected guinea pigs against both an intramuscular[247,248,280] and an aerosol challenge.[246] The licensed vaccine has also been shown to protect rhesus monkeys against an aerosol challenge.[242,270,278,282] The Centers for Disease Control and Prevention issued recommendations on the use of the anthrax vaccine in 2009.[273]

Recombinant PA is undergoing clinical trials and is considered the next-generation anthrax vaccine. Additionally, other nontoxin based vaccine approaches are being explored. These approaches include using the *B anthracis* capsule[270,279,281,283–285] and spore-specific proteins.[286–289] Although these novel antigens have been promising, it is generally agreed that PA will continue to have a prominent role in licensed anthrax vaccines.

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%[82] and 0.2% to 1.3%.[277] The vaccine licensed in the United States is reported to have a similar incidence of reactions.[290] Local reactions considered significant include induration, erythema in an area larger than 5 cm in diameter, edema, pruritus, warmth, and tenderness. These reactions peak at 1 to 2 days and usually resolve within 2 to 3 days afterward. Rare reactions include edema extending from the local site to the elbow or forearm, and a small, painless nodule that may persist for weeks. A recent study indicated that administering the vaccine over the deltoid muscle instead of the triceps can significantly reduce the frequency of local reactions.[251]

People who have recovered from a cutaneous infection with anthrax may have severe local reactions from being vaccinated.[276] Systemic reactions are characterized by flu-like symptoms, mild myalgia, arthralgia, headache, and mild-to-moderate malaise that last for 1 to 2 days. No long-term sequelae of local or systemic reactions exist and no suggestion of a high frequency or unusual pattern of serious adverse events exists.[251,256,257,291,292]

SUMMARY

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 rarely by the inhalational or gastrointestinal routes. An aerosol exposure to spores causes inhalational anthrax, which is of military concern because of its potential for use as a biological warfare agent. Aerosol exposure 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. Radiography of the chest often reveals characteristic mediastinal widening, indicating hemorrhagic mediastinitis. Hemorrhagic meningitis frequently coexists. Given the rarity of the disease and its rapid progression, it is difficult to diagnose inhalational anthrax. Treatment consists of massive doses of antibiotics and supportive care. Postexposure antibiotic prophylaxis is effective in laboratory animals and should be instituted as soon as possible after exposure. A licensed, antigen-based, nonviable vaccine is available for human use.

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