Concise Clinical Review

Anthrax Infection

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Bacillus anthracis infection is rare in developed countries. However, recent outbreaks in the United States and Europe and the potential use of the bacteria for bioterrorism have focused interest on it. Furthermore, although anthrax was known to typically occur as one of three syndromes related to entry site of (i.e., cutaneous, gastrointestinal, or inhalational), a fourth syndrome including severe soft tissue infection in injectional drug users is emerging. Although shock has been described with cutaneous anthrax, it appears much more common with gastrointestinal, inhalational (5 of 11 patients in the 2001 outbreak in the United States), and injectional anthrax. Based in part on case series, the estimated mortalities of cutaneous, gastrointestinal, inhalational, and injectional anthrax are 1%, 25 to 60%, 46%, and 33%, respectively. Nonspecific early symptomatology makes initial identification of anthrax cases difficult. Clues to anthrax infection include history of exposure to herbivore animal products, heroin use, or clustering of patients with similar respiratory symptoms concerning for a bioterrorist event. Once anthrax is suspected, the diagnosis can usually be made with Gram stain and culture from blood or surgical specimens followed by confirmatory testing (e.g., PCR or immunohistochemistry). Although antibiotic therapy (largely quinolone-based) is the mainstay of anthrax treatment, the use of adjunctive therapies such as anthrax toxin antagonists is a consideration.

Keywords: *Bacillus anthracis*; diagnosis; pathogenesis; treatment

Until recently, *Bacillus anthracis* (anthrax) infections were relatively infrequent and confined to agrarian communities in underdeveloped countries. However, the 2001 bioterrorism attack in the United States and the outbreak of anthrax infections among injectional drug users in Europe in 2009 and 2010 demonstrate that the clinical relevance of anthrax has greatly increased. Anthrax has been classified into one of three syndromes based on the primary site of infection: cutaneous, gastrointestinal, or inhalational. A fourth syndrome, characterized by severe soft tissue infections in injection drug users, has emerged. Because of the low incidence of anthrax in developed areas and the nonspecific early symptoms, many patients in these outbreaks have presented with advanced infection, which has progressed rapidly to shock. The morbidity and mortality in such patients has been high. This review discusses the microbiology of *B. anthracis*, key features of the differing syndromes, the features and potential mechanisms underlying shock with anthrax, and the diagnosis and treatment of anthrax.

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MICROBIOLOGY

B. anthracis is a gram-positive, rod-shaped bacteria that exists in the environment as a spore and can remain viable in the soil for decades (1). Spores ingested by grazing herbivores germinate within the animal to produce the virulent vegetative forms that replicate and eventually kill the host. Products (e.g., meat or hides) from infected animals serve as a reservoir for human disease. Germination from spore to vegetative organism is thought to occur inside host macrophages and is mediated by specific interactions between nutrients (primarily amino acids and purine nucleosides) and germinant nutrient receptors located on the inner membrane of the spore (2). Physiologic body temperature, as well as blood and tissue carbon dioxide levels, contribute to this process by triggering the production of important virulence factors (3). The relative ease with which large amounts of *B. anthracis* can be grown under laboratory conditions and the spore's resistance to killing and ease of dissemination facilitates the potential use of the microbe for bioterrorism (4).

After germination occurs, three factors appear key to the pathogenesis of anthrax: a capsule, the production of two toxins (i.e., lethal and edema), and the bacteria's ability to achieve high concentrations in infected hosts (5). Virulent anthrax strains carry two plasmids, pXO1 and pXO2, which encode the capsule and toxin components. The plasmids are regulated by the transcriptional factor AtxA, which is modulated by environmental factors (6). The capsule resists phagocytosis and is weakly immunogenic. Lethal toxin (LT) and edema toxin (ET) are binary toxins, each made up of two proteins: protective antigen (PA) and lethal factor (LF) for LT and PA and edema factor (EF) for ET. Protective antigen mediates cell binding and uptake of LF and EF, the toxigenic components. Lethal factor is a zinc metalloprotease that selectively inactivates mitogen-activated protein kinase kinases 1 to 4 and 6 and 7 (7). Edema factor is a calmodulin-dependent adenyl cyclase that increases intracellular cAMP levels to very high levels (8). Moayeri and Leppla provide an excellent overview of the potential actions of LF and EF in a recent review (9).

During infection, circulating PA binds to one of at least two host cellular receptors, tumor endothelial marker 8 or capillary morphogenesis gene 2, present on many tissues (Figure 1) (10). The bound PA precursor molecule undergoes furin cleavage with release of an unbound subunit. Bound PA subunits form a heptamer that one to three circulating LF and EF proteins competitively bind to. This complex undergoes endocytosis, and the toxic factors are released intracellularly.

ANTHRAX CLINICAL SYNDROMES

Cutaneous Anthrax

Ninety-five percent of reported anthrax cases are cutaneous, and most occur in Africa, Asia, and Eastern Europe where animal and worker vaccination is limited (11). It is estimated that there are approximately 2,000 cases annually worldwide (12). In the

Figure 1. Key events in the uptake of edema or lethal toxin by host cells as well as the potential effects of edema and lethal factor. During infection, circulating protective antigen (PA) binds to one of at least two host cellular receptors, anthrax tumor receptor (ATR) encoded by the tumor endothelial marker 8 gene or another receptor encoded by the capillary morphogenesis gene 2 (CMG2), both of which are present on many tissues. The bound PA precursor molecule undergoes furin cleavage with release of an unbound subunit. Bound PA subunits form a heptamer that one to three circulating lethal factor (LF) or edema factor (EF) proteins competitively bind to. This complex undergoes endocytosis, and the toxic factors are then released into the cytoplasm. Edema factor has calmodulin-dependent adenyl cyclase activity and increases intracellular cAMP levels, whereas LF inhibits mitogen activated protein kinase kinase 1 to 4, 6, and 7. These factors have been shown to influence the function of several different types of host cells, as summarized by Moayeri and Leppla (9).

2001 anthrax outbreak in the United States, cutaneous anthrax accounted for 11 of 22 cases (13). Cutaneous anthrax frequently resolves spontaneously, with the mortality rate for untreated infections estimated to be between 5 and 20% (1). With appropriate antibiotic treatment, the mortality rate is less than 1% (14). Nonetheless, cutaneous anthrax can produce shock, with one recent case series reporting an incidence of 9% (2 of 22 patients diagnosed with cutaneous anthrax) (15).

Cutaneous anthrax results when spores are introduced via breaks in exposed skin areas. The spores germinate within macrophages locally or in regional lymph nodes, and vegetative forms are released (1). The incubation period is from 1 to 12 days (1, 14). The initial skin lesion is a painless or pruritic papule associated with a disproportionate amount of edema and which progresses to a vesicular form (1–2 cm). Fever and regional lymphadenopathy can occur. The vesicle then ruptures and forms an ulcer and black eschar, which sloughs in 2 to 3 weeks (11, 14) (Figure 2). Purulence is only seen with secondary nonanthrax infection. Edema with face or neck infection may produce airway compromise (12).

Gastrointestinal Anthrax

Gastrointestinal anthrax is typically related to ingestion of spore-contaminated meat. Although gastrointestinal anthrax is uncommon, its incidence may be underestimated due to the infection's nonspecific symptomatology. The mortality rate is estimated to be 25 to 60%, and, although the rate of associated shock is not known, case reports note that severe cases are frequently complicated by shock (16, 17). The only confirmed case of gastrointestinal anthrax in the United States was atypical in that it occurred in a person who likely swallowed aerosolized anthrax

Figure 2. Cutaneous anthrax. These lesions appeared on the arms of a man who 6 days earlier had handled ill cattle. The extensive edema and hemorrhagic vesicles and bullae are typical of cutaneous anthrax and appear before the formation of a black eschar (15).

spores released from an animal-hide drum (18, 19). This patient's clinical course was complicated by shock.

There are two forms of gastrointestinal anthrax: oropharyngeal and intestinal. In oropharyngeal anthrax, spores settle in the pharyngeal area and produce ulcers. In the largest outbreak reported in 24 persons from Thailand who ate contaminated beef, the mean incubation time was 42 hours (20). Most patients presented with fever and neck swelling secondary to cervical lymphadenopathy. Initial ulcers were associated with congestion, followed by central necrosis, whitish discoloration, and eventually a pseudomembranous covering. In intestinal anthrax, spores deposit and cause ulcerative lesions anywhere from the jejunum to the cecum. The ulcers are associated with mesenteric lymphadenopathy and ascites and can lead to intestinal obstruction, bleeding, and perforation (20). Patients frequently present with nonspecific gastrointestinal symptoms (i.e., nausea, vomiting, abdominal pain, or diarrhea). In more severe cases, fever, ascites, increased abdominal girth, and shock develop (17).

In the one confirmed case in the United States, the patient initially experienced flu-like symptoms followed by nausea, vomiting, and abdominal cramps (18, 19). The patient then developed hypotension. Laboratory data were notable for leukocytosis, hemoconcentration, and an abdominal CT showing massive ascites, thickened small bowel segments, and prominent retroperitoneal lymphadenopathy (Figure 3). Exploratory laparotomy showed nodular hemorrhagic lesions in the mesentery and two areas of necrotic small bowel. The small bowel lesions and the appendix were resected. Hypotension and respiratory failure subsequently worsened. Repeat laparotomy showed a retroperitoneal hematoma that was removed. Besides antibiotics and other supportive measures, this patient received antiimmune globulin (AIG; Cangene, Winnipeg, MB, Canada). After the second surgical procedure, the patient recovered.

Inhalational Anthrax

During the 19th century, inhalational anthrax occurred in the United States and Europe among millworkers handling spore-contaminated animal hides. Industrial outbreaks are now rare due to animal vaccination, disinfecting processes, and improved factory ventilation (21, 22). Nonetheless, sporadic cases occur, especially among artisans working with animal products from endemic areas (23–25). Bioterrorism remains the greatest risk for a large-scale outbreak, as evidenced by the accidental release

Figure 3. Gastrointestinal anthrax. Coronal reconstruction images from a CT scan of the abdomen and pelvis after the administration of intravenous contrast material show a large amount of ascites and concentric wall thickening of a long segment of the distal small bowel (A, arrows). Numerous slightly enlarged lymph nodes are enhanced with intravenous contrast material and are seen at the root of the small bowel mesentery (A, arrowheads) and in the retroperitoneum (B, arrowheads) (19).

of weapons-grade anthrax in Sverdlovsk, Russia in 1979 and the 2001 outbreak in the United States (26). The mortality rate appears to have improved over time: 94% in naturally occurring cases before 1976, 86% in Sverdlovsk, and 46% in the outbreak in the United States (26, 27). Improved survival has been attributed to earlier diagnosis, better supportive care, and early and multidrug antibiotic therapy (1, 28).

Inhalational anthrax is caused by inhalation and alveolar deposition of spores less than 5 μm in size (1). Spores are phagocytosed by macrophages and carried to local mediastinal lymph nodes where they germinate into vegetative forms, replicate, and produce hemorrhagic mediastinitis (26, 29). If ineffectively treated, bacteremia and toxemia ensue, resulting in meningitis, gastrointestinal involvement, and refractory shock. Although inhalational anthrax is generally not considered an airspace disease, histology at autopsy sometimes shows focal pneumonia, possibly representing the initial site of microbial entry (26).

Inhalational anthrax has a biphasic clinical course (21, 28). After an incubation period of approximately 4 days, patients develop flu-like symptoms with fever, nonproductive cough, and myalgias lasting approximately 4 days. Without timely treatment, a second fulminant phase follows, characterized by hypotension and dyspnea. This phase may progress to death within 24 hours of its onset (21). A systematic review of 82 inhalational anthrax cases in the United States from 1900 to 2001 noted common admission findings in patients who lived ($n = 12$) or died ($n = 70$) (21, 28). Although not reported for all patients, the most frequent findings were fever or chills (92 and 62% in patients who lived or died), cough (100 and 54%), dyspnea (83 and 46%), fatigue or malaise (75 and 62%), abnormal body temperature (92 and 78%), lung findings on physical examination (100 and 74%), and tachycardia (83 and 61%). Chest radiography was consistently abnormal, most notably showing pleural effusions or a widened mediastinum in 100% of patients whether they lived or died (Figure 4). An elevated hematocrit has also been noted with inhalational anthrax. This finding, along with a widened mediastinum on radiograph and altered mental status, may help differentiate it from community-acquired pneumonia (27). A retrospective analysis of inhalational anthrax and community-

acquired pneumonia cases calculated that a derived algorithm based on these three variables was 100% sensitive (95% confidence interval, 73.5–100) and 98.3% specific (95% confidence interval, 95.1–99.6) for differentiating the two conditions.

Antibiotic treatment quickly sterilizes blood cultures with inhalational anthrax and progressive disease, and death in severely ill patients in the fulminant stage of disease has been postulated to be due ongoing toxin release (25). However, assays capable of measuring anthrax toxin components have not been sufficiently applied in conjunction with culture testing to confirm this possibility.

Injectational Anthrax

In 2001, a heroin user in Norway developed an anthrax soft tissue infection resulting from a subcutaneous drug injection. Infection was complicated by septic shock, meningitis, and death despite therapy. This syndrome, which appeared different from cutaneous disease, was referred to as “injectational anthrax” (30). A major outbreak of injectational anthrax has recently occurred in the United Kingdom (47 recognized cases and 13 deaths in Scotland, 5 cases and 4 deaths in England) primarily in subcutaneous or intramuscular heroin users (31, 32). Two cases (and one death) have been noted in Germany (33, 34). Important differences between cutaneous and injectational anthrax have included an increased risk of shock and a higher mortality rate despite antibiotic therapy (< 1% versus 34%, respectively). There were several potential sources for contamination of this heroin. Most heroin sold in Europe originates in Afghanistan and is transported through Iran and Turkey—all countries where anthrax is enzootic (35, 36). Moreover, heroin is routinely cut by 50 to 99% with diluents before and after it reaches the consumer. It is estimated that 61 to 68% of street heroin samples in the United States are contaminated with pathogens, most frequently nonanthrax *Bacillus* species (37).

It is believed that in injectational anthrax, spores germinate at the inoculation site, and the bacteria’s capsule facilitates local spread. Whether disseminated disease results from intravascular injection of spores or spread of local soft tissue infection is unknown. Significant edema at the injection site is common, but, in contrast to cutaneous disease, papules, vesicles, and eschars are not typically observed (30, 38) (Figure 5). Excessive bruising at the injection site may occur early. Such symptoms are common in intravenous drug users. Differences in these early symptoms and the impetus to seek medical attention comparing cutaneous and injectational anthrax may provide a basis for the poorer observed prognosis with the latter. Alternatively, injectational anthrax may involve the delivery of a larger and more concentrated bacterial inoculum to a deeper site. Surgical exploration of wounds from injectational anthrax cases has revealed prominent tissue edema, diffuse capillary bleeding, and necrosis of the superficial adipose tissue. These lesions differed from abscesses that contain purulent material and from necrotizing fasciitis, which involves deeper soft tissue and is characterized by microvascular thrombosis and turbid fluid (38). Severe cases developed thrombocytopenia and coagulopathy. After initial hemodynamic stabilization, a notable number of patients have developed recurrent shock resistant to therapy.

SHOCK DURING ANTHRAX INFECTION

Clinical Characteristics

Although shock is rare with cutaneous anthrax, it occurs commonly with inhalational disease (5/11 cases in the 2001 outbreak in the United States) (13, 39). The rates of shock associated with gastrointestinal or injectational anthrax are not well defined but

Figure 4. Inhalational anthrax. (A and B) The contrast-enhanced chest CT scans from a patient with inhalational anthrax shows perihilar parenchymal lung disease (A, arrow), widening mediastinum, hilar adenopathy, pleural effusions, and peribronchial infiltrates as well as patchy peribronchial air-space disease, especially on the right (B, arrow). (C–E) Photomicrographs of histopathologic specimens of hilar soft tissue from the same patient at autopsy shows perivascular and peribronchial hemorrhage (C, arrow; H&E; original magnification: $\times 10$), hemorrhage and necrosis (D, arrow; H&E; original magnification: $\times 20$), and abundant gram-positive bacilli (E, arrow; Brown-Brenn; original magnification: $\times 100$) (84).

also appear substantial (20, 38). The recent confirmed case of gastrointestinal anthrax in the United States developed severe shock. In preliminary reports from the experience with injectional anthrax in the United Kingdom, shock was also a notable finding. There are few invasive systemic or myocardial hemodynamic measures available clinically to help characterize the pathophysiologic mechanisms underlying anthrax-associated shock. For example, to what extent peripheral vascular versus myocardial dysfunction contributes is unknown clinically.

Nonetheless, there are a number of unique clinical features that have been noted. Although hemoconcentration is rare in sepsis due to more common pathogens, it has been frequently noted with anthrax-associated sepsis (15, 19, 27). This finding is likely related in part to fluid shifts. Soft tissue edema is a hallmark of cutaneous anthrax and can extend well beyond the site of infection. Also, fluid collections in the chest or abdomen are typical of inhalational and gastrointestinal anthrax, respectively. The fact that liberal fluid resuscitation (> 10 L in 24 h) and

Figure 5. Injectional anthrax. Preoperative photographs of a woman (A) with skin necrosis involving the medial aspect of her left thigh and labia majora and a man (B) with swelling of his scrotum and skin necrosis involving his buttock (85). (C) Surgical debridement of necrotic skin and fascia of a patient with injectional anthrax and compartment syndrome of the right arm (76).

extensive soft tissue edema have been described with injectional anthrax locally and in areas distant from the infection site suggests that extravasation of fluid also occurs with this syndrome (38). Hyponatremia has also been a frequent finding in inhalational anthrax and was described in 8 of 10 cases in the 2001 outbreak in the United States (40). How often this occurred before as opposed to after fluid resuscitation is not known.

Another notable difference from other types of bacteria is that once anthrax infection progresses to septic shock, it appears to be very resistant to conventional supportive measures (38). In the 2001 outbreak in the United States, the five patients reported to develop hemodynamic instability died despite aggressive support. The mortality rate, specifically in patients with shock in the injectional anthrax outbreak in the United Kingdom, has not been reported. Because some of the patients did not require ICU care, the overall number of deaths described (18/53) suggests a high mortality rate among those patients who required intensive care therapies.

Potential Pathogenic Mediators

Although clinical observations are limited, preclinical studies provide insight into potential mechanisms underlying shock during anthrax. Several bacterial components may contribute to this. However, how the actions of these components interact is unclear.

Lethal toxin. *In vivo* studies since the outbreak in the United States have shown that LT may play an important role in shock during anthrax infection and that its mechanisms of action are likely multifactorial. In rats, 24-hour LT infusions simulating toxin release during infection produced progressive hypotension, hemoconcentration, and increased lactate (41). Similar LT infusions in sedated, instrumented, and mechanically ventilated canines caused progressive shock associated with reductions in central venous pressure that persisted in nonsurvivors and were associated with lactic acidosis (42). These and other findings have suggested that LT may alter peripheral vasculature function. Consistent with this, *in vitro* LT disrupts endothelial cell function through stimulation of endothelial cell apoptosis and alterations in actin fibers and via mast cell activation (10, 43–45). Pulmonary dysfunction has not been prominent in these LT-challenged models. However, LT may also directly depress myocardial function. LT injection in rats and a limited number of canines reduced left ventricular function when measured 18 or 96 hours later, respectively (46). In mice, LT challenge reduced left ventricular ejection fraction (LVEF) as early as 6 hours (9). In another canine model, 24-hour LT infusions caused progressive reductions in LVEF from 48 to 96 hours but not in pulmonary artery occlusion pressures (42). Consistent with these *in vivo* findings, LT depressed cardiac myocyte function *in vitro* via NADPH oxidase-mediated mechanisms (47). Finally, it is possible that LT has a direct effect on mitochondrial or other function that injures cells. In rats, a regimen of norepinephrine infusion, which improved survival in LPS-challenged animals, increased blood pressure but not survival with LT (48).

Edema toxin. Although on a molar weight basis ET is less lethal than LT, it may play an important role in shock with anthrax. Clinically, substantial extravasation of fluid with severe anthrax cases has implicated ET in systemic infection (13). EF is known to increase intracellular cAMP to very high levels (8). cAMP in turn plays a central role in stimulating vasodilation (49, 50). In canines, 24-hour ET infusions rapidly reduced central venous arterial and blood pressure in patterns persisting for up to 96 hours (42). These changes were not associated with hemoconcentration or extravasation of fluid, suggesting that reductions in CVP and hypotension might represent direct vasodilation.

ET also produced marked tachycardia, consistent with increased cAMP in cardiac pacemaker cells, but did not alter LVEF (51). Finally, despite hypotension, ET produced 10-fold increases in urine output as well as hyponatremia, suggesting renal tubular dysfunction (42). In rats administered ET, echocardiography demonstrated reduced preload, and lung pathology did not show extravasation of fluid (46). In a perfused rat heart model, ET increased heart rate and coronary flow, the latter once again consistent with direct vasodilation. ET also increased myocardial tissue and effluent cAMP levels (52). Monoclonal antibody directed against PA or adenylylase, a nucleoside that inhibits EF adenylylase activity, inhibited these changes. ETs could also potentially contribute to vasopressor-resistant shock because agents that stimulate intracellular cAMP also blunt the effects of vasopressors.

Cell wall. Although lethal LT or ET challenges do not stimulate inflammatory mediator release, live *B. anthracis* challenge does, and this may contribute to shock and organ injury with anthrax (53, 54). Emerging evidence suggests that peptidoglycan from anthrax cell wall contributes to this inflammatory response. Whole anthrax cell wall was shown originally to stimulate TNF- α , IL-1 β , and IL-6 release from human peripheral blood mononuclear cells (55). A subsequent study suggested that this activity was related to stimulation of cell surface TLR2/6 heterodimers (56). Purification of cell wall showed that its immunostimulatory effect was primarily related to peptidoglycan (57). Peptidoglycan was also shown to be shed by replicating bacteria. *In vitro* infection with anthrax also results in NOD-2-dependent IL-1 β release (58). Challenge with whole anthrax cell wall in rats produced dose-dependent increases in lethality, lactate and circulating inflammatory cytokines, chemokine and nitric oxide levels, and thrombocytopenia (59). Thus, anthrax cell wall could produce a robust intravascular inflammatory response and hemodynamic and organ dysfunction. Patients and animals dying with anthrax have very high bacterial loads, the breakdown of which could contribute to shock (56, 57).

Proteases. *B. anthracis* produces proteases other than LF that may contribute to shock and tissue injury. In culture studies, the delta Ames (pXO1⁺ and pXO2⁺) anthrax strain produced metalloproteases belonging to the M4 thermolysin and M9 bacterial collagenase families (60). Purified preparations of these proteases produced hemorrhagic tissue injury in mice. Furthermore, chemical inhibitors or immune serum against the metalloproteases improved survival in animals challenged with Sterne strain spores. Additional studies of such proteases have implicated them in other pathophysiologic effects (61).

Secondary changes with anthrax infection that may contribute to shock. Although anthrax produces several components potentially contributing to shock and organ injury, secondary changes may complicate this picture. For example, lymph node involvement may disrupt lymphatic function and contribute to fluid extravasation noted clinically. Intestinal perforation with gastrointestinal anthrax and tissue breakdown or infection with nonanthrax strains in injectional anthrax may also complicate shock and tissue injury. Differentiating primary from secondary mechanisms in such patients may be difficult.

DIAGNOSTIC METHODS

Based on CDC recommendations, a confirmed anthrax case is defined as a clinically compatible one with isolation of *B. anthracis* or with at least two positive supportive tests using serologic or other methods (Table 1) (62). Routine culture with confirmation by immunohistochemical staining or real-time PCR is most frequently employed for diagnosis. There is a range of other tests that can be used to identify the bacteria or the toxin

TABLE 1. SUMMARY OF DIAGNOSTIC AND SUPPORTIVE TESTS EMPLOYED TO CONFIRM THE DIAGNOSIS OF ANTHRAX IN CLINICALLY COMPATIBLE CASES

Test	Description
Routine culture	Morphology, hemolysis, motility, and sporulation are evaluated after plating suspected <i>Bacillus anthracis</i> cells on standard 5% sheep blood or chocolate agar. <i>B. anthracis</i> colonies will be 2–5 mm in diameter with irregularly round borders and a ground glass appearance, are nonhemolytic and nonmotile, and should sporulate 18–24 h after incubation at 35–37°C in a non-CO ₂ environment
Immunohistochemical staining (Gram stain)	Detection of <i>B. anthracis</i> cells in formalin-fixed tissues using antibodies specific for cell wall or capsule antigens
Real-time PCR	Targets three distinct loci on the <i>B. anthracis</i> chromosome and each of the two virulence plasmids
Capsule staining	India ink, McFadyean staining, or direct fluorescence assay may be used to visualize encapsulated <i>B. anthracis</i> in culture or directly in clinical specimens
Susceptibility to gamma phage lysis	Gamma phage specifically lyses <i>B. anthracis</i> vegetative cells and can be used to confirm diagnosis for isolates with a concomitant positive capsule stain
Time-resolved fluorescence	Anti-PA assay similar to ELISA but that uses detector antibodies labeled with fluorescing lanthanide chelates instead of enzymes and pulses of excitation energy to measure fluorescence
Immunochromatography (Redline Alert)	A lateral flow immunoassay containing a monoclonal antibody that is specific for the presence of a cell surface protein found in <i>B. anthracis</i> vegetative cells and used for rapid presumptive identification of <i>B. anthracis</i> from nonhemolytic <i>Bacillus</i> colonies cultured on sheep blood agar plates
Direct fluorescent assay	Two-component assay that uses fluorescein-labeled monoclonal antibodies to detect the galactose/N-acetylglucosamine cell-wall-associated polysaccharide and capsule produced by <i>B. anthracis</i> vegetative cells in culture or directly in clinical specimens
Fluorescence resonance energy transfer assay	Fluorogenic peptide-based assay used to screen for <i>B. anthracis</i> lethal factor protease activity
Europium nanoparticle-based immunoassay	Nanoparticle-based detection of antibody response against the protective antigen anthrax toxin protein
Electrophoretic immunotransblot reaction	Measures antibody protective antigen and/or lethal factor bands in serum from patients with suspected <i>B. anthracis</i> infection
Quantitative human anti-PA IgG ELISA	Enzyme-based colorimetric detection of antibody response against the protective antigen (PA) anthrax toxin protein
Molecular characterization	Multi-locus variable-number tandem repeat analysis and sequencing of genes coding for 16S ribosomal RNA may be conducted for species identification and molecular characterization <i>B. anthracis</i> isolates

components (Table 1). *B. anthracis* can be isolated from numerous clinical samples, including blood, skin lesion exudates, cerebrospinal fluid, pleural fluid, sputum, and feces. However, prior antimicrobial therapy greatly reduces this sensitivity. Appropriate therapy can result in negative blood cultures within 6 to 12 hours of administration, although the exact frequency with which this occurs is unknown. Anthrax should be considered immediately if Gram stain of specimens reveals gram-positive bacilli growing in chains (63). Suspected isolates should be sent to a Laboratory Response Network reference laboratory for testing, and local or state health departments should be notified (64, 65). Laboratories in the Laboratory Response Network provide the supportive testing necessary to confirm an anthrax diagnosis.

MANAGEMENT

Active Disease

Given the potential severity of anthrax infection, the first suspicion of disease must prompt antibiotic treatment pending confirmed diagnosis (4). *B. anthracis* is susceptible to a variety of antimicrobial agents, including penicillin, chloramphenicol, tetracycline,

erythromycin, streptomycin, fluoroquinolones, and cefazolin, along with other first-generation cephalosporins. Anthrax is resistant to many later-generation cephalosporins, such as cefuroxime, cefotaxime, ceftazidime, aztreonam, and trimethoprim-sulfamethoxazole (66). Table 2 summarizes the empiric antibiotic treatment recommendations for patients with suspected anthrax infection. Although antibiotics are important in the management of *B. anthracis*, their efficacy might not only be due to bacterial clearance. It is possible that agents such as clindamycin used in the 2001 outbreak also inhibit protein or RNA synthesis necessary for toxin production (67). However, while this has been shown for some types of gram-positive bacteria, it has not been demonstrated for anthrax. Aside from antimicrobial therapy, there are a number of potential adjunctive interventions for active anthrax: agents designed to directly inhibit toxin, thoracentesis to remove a potential reservoir of toxin, and the use of glucocorticoids. Before antimicrobials, passive immunization with antiserum was used to treat anthrax. A recent systematic review of inhalational anthrax suggested that antiserum reduced mortality (28). However, this finding may be confounded by improvements in antibiotic and other supportive measures. In the United States, two antibody preparations are potentially

TABLE 2. ANTIBIOTIC MANAGEMENT FOR ADULTS WITH ANTHRAX INFECTION

Clinical Syndrome	Initial Therapy*†‡	Comment
Cutaneous	Ciprofloxacin 500 mg orally twice daily or doxycycline 100 mg orally twice daily for 60 d	If systemic illness or extensive edema involving face or neck present, then treat using regimen for inhalational anthrax
Inhalational, gastrointestinal, or injectional	Ciprofloxacin 400 mg intravenously every 8 h or doxycycline 100 mg intravenously every 12 h combined with second agent: clindamycin 600 mg intravenously every 8 h or penicillin G 4 MU every 4–6 h or meropenem 1 gm intravenously every 6–8 h or rifampin 300 mg every 12 h	Clindamycin recommended for possible role in preventing toxin production. If meningitis is suspected, then ciprofloxacin is favored over doxycycline, and penicillin or rifampin should be administered

* Treatment for 60 d is recommended to avoid relapse or breakthrough of incubating disease. If initial therapy is intravenous, then convert to oral administration (ciprofloxacin or doxycycline) when clinically indicated.

† Steroids may be considered as an adjunct therapy for patients with severe edema and for meningitis.

‡ For pregnant women, avoid doxycycline. Use ciprofloxacin and switch to oral penicillin once susceptibilities are known.

TABLE 3. HEALTH CARE AND INTENSIVE CARE UNIT CONSIDERATIONS

Use standard barrier precautions. High-efficiency particulate air filter masks or other measures for airborne protection are not required.
Use contact isolation precautions for patients with draining anthrax lesions.
Immunization or postexposure prophylaxis is not required for healthcare workers or household contacts unless exposed to an aerosol or surface contamination.
Dressings from draining anthrax lesions should be disposed of as biohazardous waste.
Individuals coming in direct contact with a substance potentially containing <i>Bacillus anthracis</i> should wash exposed skin and clothing thoroughly with soap and water.
A standard hospital disinfectant (e.g., hypochlorite) is adequate to clean environmental surfaces potentially contaminated with infected body fluids.
Human and animal remains infected with <i>B. anthracis</i> should be burned.

available. One is derived from individuals previously vaccinated with anthrax vaccine adsorbed (AVA) vaccine and is termed anthrax immune globulin (AIG) (Cangene, Winnipeg, MB, Canada). Treatment with AIG was associated with survival in one of two recent cases of severe inhalational anthrax as well as in a case of gastrointestinal anthrax (25, 68). Several patients in the injectional anthrax outbreak in the United Kingdom received AIG, although the results have not been reported. AIG is available from the CDC (69). The second preparation is a human monoclonal antibody generated against recombinant PA, raxibacumab (ABthrax; Human Genome Science, Rockville, MD). This antibody improved outcome in lethal toxin-challenged or spore-challenged animal models and appears to be safe in healthy humans (70, 71).

Pleural fluid drainage may be important in the management of inhalational anthrax cases to improve respiratory function and to remove a potential toxin reservoir. This intervention was potentially effective in several recent cases of inhalational anthrax as well as in a review of cases from 1900 to 2005 (25, 28, 72). In this latter review of 70 cases, 10 of 12 surviving patients received pleural fluid drainage along with other therapy.

Based upon case reports, glucocorticoids may serve as an adjunctive therapy for patients with cutaneous anthrax and extensive edema involving the head and neck (39, 73, 74). Glucocorticoid therapy can also be considered in patients with meningoencephalitis (94% mortality rate in one large case series); however, this recommendation is based solely on the clinical experience of treating meningitis of other bacterial etiologies (4, 75).

Although the management of injectional anthrax is evolving, aggressive surgery with debridement may be necessary when there is a clinical need to control soft tissue infection (38, 76). Drainage of extravascular fluid collections when present may also be important.

Postexposure Prophylaxis

A recent CDC conference on anthrax postexposure prophylaxis recommended treatment with 60 days of oral ciprofloxacin or doxycycline as equivalent first-line agents (77). In addition to oral antimicrobial therapy, the CDC calls for treatment of adults with AVA (Biothrax, Emergent Biosolutions, Rockville, MD) administered at time 0 and at 2 and 4 weeks (78). AVA and anthrax vaccine precipitated (Health Protection Agency, Porton Down, UK) are aluminum hydroxide-precipitated preparations of PA from attenuated, nonencapsulated *B. anthracis* Sterne strain (79, 80). AVA is licensed by the FDA for preexposure prophylaxis against inhalational anthrax in persons at occupational risk of disease, although it has not been studied clinically (81). In nonhuman primates challenged with aerosolized *B. anthracis* spores, animals that received postexposure vaccination with AVA in addition to antibiotics had a greater survival rate when rechallenged with anthrax (82). Guidelines continue to recommend AVA for postexposure prophylaxis along with antibiotics for 60 days because the vaccine may provide benefits and appears to have an excellent safety profile (4). Although local reactions at the time of vaccination are

described, long-term adverse events have not been clearly documented (83).

Infection Control Considerations

Because there are no documented cases of person-to-person transmission of anthrax (including during the 2001 anthrax attack), respiratory isolation for hospitalized patients is not required. Additional details regarding appropriate infection control measures and anthrax are outlined elsewhere (Table 3) (4).

CONCLUSIONS

Although anthrax infection is rare in developed countries, the potential for large outbreaks persists, whether related to bioterrorism or injectional drug use. Its infrequency and nonspecific early symptomatology suggest that, in the event of an outbreak, many patients may present with advanced disease, which has proven difficult to treat. Further defining the mechanisms underlying later-stage anthrax and developing effective management strategies that can be administered on a broad scale are necessary.

Author Disclosures are available with the text of this article at www.atsjournals.org.

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