REVIEW ARTICLE

Anthrax as a Potential Biological Warfare Agent

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Anthrax is a zoonotic illness recognized since antiquity. Today, human anthrax has been all but eradicated from the industrialized world, with the vast majority of practitioners in the United States unlikely to have seen a case. Unfortunately, the disease remains endemic in many areas of the world, and anthrax poses a threat as a mass casualty–producing weapon if used in a biological warfare capacity. *Arch Intern Med. 1998;158:429-434*

Anthrax has been described for millennia, beginning with the fifth Egyptian plague (circa 1500 BC). Virgil recorded a lyrical description of anthrax in 25 BC, and the disease became known during the Middle Ages as the "Black Bane."[1,2] In the 1870s, Robert Koch demonstrated for the first time the bacterial origin of a specific disease, with his studies on experimental anthrax, and also discovered the spore stage that allows persistence of the organism in the environment. Shortly afterward, John Bell recognized *Bacillus anthracis* as the cause of woolsorter disease (inhalational anthrax), and was instrumental in establishing wool disinfection procedures. The disinfection measures proved effective in reducing the incidence of woolsorter disease, and they became standard in the British woolen industry.[3,4] William Greenfield's successful immunization of livestock against anthrax soon followed in 1880, although Louis Pasteur's 1881 trial of a heat-cured anthrax vaccine in sheep is usually remembered as the initial use of a live vaccine.[5]

EPIDEMIOLOGY

Livestock

Anthrax is a disease of herbivores, with sheep, goats, cattle, and, to a lesser degree, swine typically infected. Gastrointestinal anthrax with subsequent systemic dissemination is acquired by livestock after grazing on forage plants contaminated by spores. Anthrax may persist in the environment for many years after contamination of a pasture. Environmental persistence appears to be related to a number of factors, including high levels of soil nitrogen and organic content, a pH level higher than 6.0, and ambient temperature higher than 15°C. Drought or heavy rains trigger spore germination and bacterial multiplication, which also appear important in maintaining the organism in potentially infectious quantities.[6] Blowflies and vultures have been implicated in the persistence and spread of anthrax in Africa.[7]

Once prevalent in nearly all areas where livestock were raised, intensive animal vaccination programs have now restricted anthrax mainly to Africa and Asia. Sporadic outbreaks still occur in many other countries including the United States, where an "anthrax belt" extends across the Great Plains.[8] Incidence of the disease has actually increased in Africa in recent years, prompting the World Health Organization to seek to improve surveillance and control efforts. An effective live spore vaccine is marketed by a South African firm for 10 cents per dose (1994 cost), but vaccination in the developing world remains spotty.[9]

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Human

Human cases of anthrax have traditionally fallen into 2 categories, either agricultural or industrial. Agricultural cases consist of laborers in direct contact with infected animals (herders, butchers, and slaughterhouse workers), and industrial cases involve individuals in contact with infected animal products, in particular workers in animal hair processing mills and those handling bonemeal. Not surprisingly, cases in the developed world have tended to be of the industrial variety. Glassman[10] estimated that the worldwide incidence of anthrax in 1958 was between 20 000 and 100 000 cases annually. The incidence may be considerably lower today; however, anthrax is not a reportable disease in more than half of African nations,[9] and the true frequency of the disease is unknown. In the United States, the total annual incidence has fallen from an average of nearly 130 cases in the early part of the 20th century to less than 1 case annually over the past 2 decades. Of the 235 cases reported between 1955 and 1994, 224 were cutaneous and 11 inhalational, and 20 were fatal.[8,11] Most of the US cases in recent decades have resulted from exposure to wool or animal hair.[12]

Human cases are invariably zoonotic in origin, with no convincing data to suggest that human-to-human transmission has ever taken place. Primary disease takes 1 of 3 forms. Cutaneous, the most common, results from contact with an infected animal or animal products. Inhalational is much less common and a result of spore deposition in the lungs, while gastrointestinal is due to ingestion of infected meat. Most literature cites cutaneous disease as constituting 95% of cases, with inhalational disease responsible for 5% and the gastrointestinal form for 0% to 5%; however, the incidence of inhalational anthrax in less industrialized nations is probably lower. Gastrointestinal disease, which has never been reported in the United States, may be more common in the developing world.

Epidemics of human anthrax have been reported, with the 2 most completely described outbreaks from Zimbabwe in 1978 through 1980, and from Sverdlovsk, in the former Soviet Union, in 1979.[13-19] The Zimbabwe epidemic followed on the heels of a cattle outbreak that arose after the breakdown of veterinary care during the Rhodesian civil war. Thousands of human cases resulted, with 1 province alone reporting nearly 6500 infections (virtually all cutaneous), with approximately 100 fatalities.[15-17] The Sverdlovsk (now Yekaterinburg) incident occurred in April 1979 in an industrial city of 1.2 million people just east of the Ural Mountains. Eventually, admissions were made that deaths were due to inhalational anthrax, the result of a mishap at a military microbiology facility, and not to gastrointestinal anthrax as was originally claimed.[18,19] At least 66 deaths occurred in a 4-km swath downwind from the incident, and the details of autopsies performed on 42 patients were eventually published by 2 of the pathologists involved.[14,18] Of interest, the youngest patient known to have been infected in the incident was 24 years old, and a large series of inhalational anthrax cases from southwest Russia earlier this century was also remarkable for a near-total absence of children.[13] Consequently, it has been suggested that the inhalational form of the disease may have a predilection for older patients, although this remains unproven.

MICROBIOLOGY

Bacillus anthracis is a large (1-1.5 ×3-10 µm) gram-positive sporulating rod, with square or concave ends. Growing readily on sheep blood agar, *B anthracis* forms rough gray-white colonies of 4 to 5 mm, with characteristic comma-shaped or "comet-tail" protrusions. Several tests are helpful in differentiating *B anthracis* from other *Bacillus* species. *Bacillus anthracis* is characterized by an absence of the following: hemolysis, motility, growth on phenylethyl alcohol blood agar, gelatin hydrolysis, and salicin fermentation. In the United States, *Bacillus* isolates lacking these characteristics and having morphological features on Gram staining consistent with *B anthracis* should be submitted to the Centers for Disease Control and Prevention via the state laboratory. *Bacillus anthracis* may also be identified by the API-20E and API-50CHB systems used in conjunction with the previously mentioned biochemical tests.[20,21] Definitive identification is based on immunological demonstration of the production of protein toxin components and the poly-D-glutamic acid capsule, susceptibility to a specific bacteriophage, and virulence for mice and guinea pigs.

PATHOGENESIS

The virulence of *B anthracis* is dependent on 2 toxins, lethal toxin and edema toxin, as well as on the bacterial capsule. The importance of a toxin in pathogenesis was demonstrated in the early 1950s, when sterile plasma from anthrax-infected guinea pigs caused disease when injected into other animals.[22] Efforts since have shown the anthrax toxins to be composed of 3 entities, which in concert lead to some of the clinical effects of anthrax.[23,24] The first of these, protective antigen, is an 83-kd protein so named because it is the main protective constituent of anthrax vaccines.[25] The protective antigen binds to target cell receptors and is then proteolytically shorn of a 20-kd fragment. A second binding domain is then exposed, which combines with either edema factor, an 89-kd protein, to form edema toxin, or lethal factor, a 90-kd protein, to form lethal toxin.[26] The respective toxins are then transported across the cell membrane, and the factors are released into the cytosol where they exert their effects. Edema factor, a calmodulin-dependent adenylate cyclase, acts by converting adenosine triphosphate to cyclic adenosine monophosphate. Intracellular cyclic adenosine monophosphate levels are thereby increased, leading to the edema characteristic of the disease.[27] The action of lethal factor, believed to be a metalloprotease, is less understood. Work in recent years has shown edema toxin to inhibit neutrophil phagocytosis.[28] Lethal toxin has been demonstrated at high concentration to lyse

macrophages, while inducing the release of tumor necrosis factor and interleukin 1 at lower concentrations.[29,30]

Hanna and colleagues[29] recently showed that a combination of antibodies to interleukin 1 and tumor necrosis factor was protective against a lethal challenge of anthrax toxin in mice, as was the human interleukin 1 receptor antagonist. Macrophage-depleted mice were shown to resist lethal toxin challenge, but to succumb when macrophages were reconstituted. The importance of the poly-D-glutamic acid bacterial capsule, the other major virulence determinant, was demonstrated in experiments early in the 20th century in which an unencapsulated strain resulted in attenuation.[31] Presumably, the capsule enhances virulence by preventing phagocytosis, and perhaps by preventing lysis of the organism by cationic host proteins.[32] The genes for both the toxin and the capsule are known to be encoded by plasmids, designated pXO1[33] and pXO2, respectively.[34,35]

Disease occurs when spores enter the body, germinate to the bacillary form, and multiply. In cutaneous disease spores gain entry through cuts, abrasions, or in some cases through certain species of biting flies.[2,15-17] Germination is thought to take place in macrophages, and toxin release results in edema and tissue necrosis but little or no purulence, probably because of inhibitory effects of the toxins on leukocytes. Generally, cutaneous disease remains localized, although if untreated it may become systemic in 5% to 20% of cases, with dissemination via the lymphatics.[36] In the gastrointestinal form, *B anthracis* is ingested in spore-contaminated meat, and may invade anywhere in the gastrointestinal tract. Transport to mesenteric or other regional lymph nodes and replication occur, resulting in dissemination, bacteremia, and a high mortality rate. Very little pathologic correlation is available for this unusual form of the disease; however, autopsies from Russian cases earlier this century suggest that the initial site of infection is most commonly the terminal ileum or cecum.[13] As in other forms of anthrax, involved nodes show an impressive degree of hemorrhage and necrosis.

The pathogenesis of inhalational anthrax is better studied and understood. Inhaled spores are ingested by pulmonary macrophages and carried to hilar and mediastinal lymph nodes, where they germinate and multiply, elaborating toxins and overwhelming the clearance ability of the regional nodes.[37,38] Bacteremia occurs, and death soon follows. A significant inoculum of spores is necessary for disease to develop; one study in mill workers found that unvaccinated subjects inhaled between 140 and 690 anthrax spores of 5 µm or less (ie, potentially pathogenic) per day without apparent ill effects.[39] Another study of healthy workers in a goat hair processing factory found that 14 of 101 subjects had *B anthracis* isolated from either the nose or the pharynx.[40] The minimum infectious inhaled dose for humans is unknown, and in nonhuman primate studies it has ranged from approximately 4000 to 80 000 spores.[41]

Fritz and colleagues[38] in their autopsy study of inhalational anthrax in rhesus monkeys found hemorrhagic, edematous lymph nodes not only in the mediastinum, but also in the mesenteric, axillary, and inguinal chains, reinforcing the systemic nature of inhalational disease. Some of the monkeys were also found to have hemorrhagic changes in the meninges, lung parenchyma, gastrointestinal tract, pancreas, myocardium, and kidneys. All monkeys tested had heavy loads of *B anthracis* in the blood at the time of death. In an earlier study in rhesus monkeys, intrathoracic nodes were more frequently involved than other lymph nodes.[42] Similar findings were reported in the Sverdlovsk autopsy series.[14] The latter study also shed more light on the inhalational form of the disease, and underscored the systemic nature of the process. Subjects of all 42 autopsies had severe hemorrhagic mediastinitis and lymphadenitis, with 11 showing focal hemorrhagic, necrotizing pneumonia at what was thought likely to be the portal of entry. All but 3 of the cases had evidence of submucosal gastrointestinal tract involvement consistent with hematogenous spread. Hemorrhagic lymphadenitis of the mesenteric nodes was evidenced in 9 of 42 subjects, and the well-recognized propensity of *B anthracis* to cross the blood-brain barrier was emphasized, with fully half demonstrating a hemorrhagic meningitis. The pathologists stressed the remarkable degree of edema seen in their cases, particularly in the leptomeningeal and pulmonary areas, with pleural effusions and a gelatinous mediastinitis commonly found.[14]

CLINICAL MANIFESTATIONS

Cutaneous disease develops an average of 2 to 5 days after exposure (range, 12 hours to 5 days in Gold's series of 117 cases),[43] beginning as a nondescript papule that during the next 24 to 48 hours becomes vesicular, usually 1 to 2 cm in diameter. A striking degree of edema surrounding the lesion is typical, and lesions on the head and neck have a propensity for impressive presentations, on occasion leading to airway compromise.[44] *Bacillus anthracis* is easily isolated from the vesicular fluid and visible on Gram staining at this stage, although neutrophils are conspicuously absent. The lesion, which is sometimes pruritic but not painful, generally ruptures near the end of the first week, leaving an ulcer that progresses to the characteristic black eschar that gave the disease its name (anthrax is derived from the Greek word for coal). The eschar generally sloughs 2 to 3 weeks after appearance. Many patients exhibit fever, headache, malaise, and regional lymphadenopathy. Differential diagnosis includes tularemia, plague, cutaneous diphtheria, staphylococcal disease, rickettsial infection, and orf, a viral disease of livestock. Recovery is the rule, although a fatality rate of 5% to 20% in untreated disease is frequently cited, due to dissemination of disease and resulting septicemia. Mortality in treated patients is less than 1% (1 of 117 patients in Gold's series).[43] Although use of antibiotics prevents dissemination, they do not affect the natural history of the lesion, which progresses through the described sequence despite therapy.

Gastrointestinal anthrax begins 2 to 5 days after ingesting contaminated meat, and has been described in 2 rare forms. One form presents as severe abdominal pain, hematemesis, melena and/or hematochezia, ascites, and on occasion profuse, watery diarrhea.[8,44] Mortality is high, and the disease is difficult to diagnose antemortem except in an epidemic setting. An oropharyngeal variant has also been described, with a 1982 outbreak involving 24 patients in Thailand secondary to eating infected cattle and water buffalo. All had marked neck swelling, most had ulcerative lesions of the oropharynx, and 3 of the 24 died.[45,46]

Inhalational anthrax begins after a 1- to 6-day incubation period following exposure. A nonspecific syndrome consisting of low-grade fever, nonproductive cough, myalgias, and malaise is initially present, with transient improvement in some patients after 2 to 4 days. Abrupt onset of respiratory distress ensues, with shock and death typically following in less than 24 hours. The initial phase is essentially impossible to diagnose in the absence of a known outbreak. Advanced disease may be suspected on the basis of the characteristically widened mediastinum and pleural effusions despite otherwise normal chest x-ray findings. Historically, inhalational anthrax was considered uniformly fatal; however, this was based on case reports prior to the advent of intensive care unit treatment, and there were at least 11 survivors in the Sverdlovsk incident.[18] As in the Sverdlovsk experience, systemic anthrax is complicated by meningitis in up to 50% of cases, is usually bloody, and is sometimes associated with subarachnoid hemorrhage.[47]

TREATMENT

Penicillin remains the drug of choice for treatment of susceptible strains of anthrax, with ciprofloxacin and doxycycline suitable alternatives. Some data in experimental models of infection suggest that the addition of streptomycin to penicillin may be helpful. Penicillin resistance remains extremely rare in naturally occurring strains[48]; however, the possibility of resistance should be suspected in a biological warfare attack. Cutaneous anthrax may be treated orally, while gastrointestinal or inhalational disease should receive high doses of intravenous antibiotics (penicillin G, 4 million units every 4 hours; ciprofloxacin, 400 mg every 12 hours; or doxycycline hyclate, 100 mg every 12 hours). The more severe forms will require intensive supportive care and have a high mortality rate despite optimal therapy. The use of antianthrax serum, while no longer available for human use except in the former Soviet Union, was thought to be of some use in the preantibiotic era, although no controlled studies were performed.[49] Antitoxin was reportedly used in the Sverdlovsk epidemic.[18] Reconsideration of the use of antitoxin in cases of systemic anthrax seems reasonable, along with development of cytokine-modulating agents.[29]

VACCINES

Although anthrax vaccination dates to the early studies of Greenfield and Pasteur, the "modern" era of anthrax vaccine development began with Sterne's work with a toxin-producing, unencapsulated (attenuated) strain in the 1930s. Administered to livestock as a single dose with a yearly booster, the vaccine was highly immunogenic and well tolerated in most species, although somewhat virulent in goats and llamas. This preparation is essentially the same as that administered to livestock around the world today.[50] The first human vaccine was developed in 1943 at the Soviets' Sanitary Technical Institute from nonencapsulated strains. This live spore vaccine, similar to Sterne's product, is administered by scarification with a yearly booster. Soviet studies show a reduced risk of 5- to 15-fold in occupationally exposed workers.[51]

The British and US vaccines were developed in the 1950s and early 1960s, with the US product an aluminum hydroxide–adsorbed cell-free culture filtrate of an unencapsulated strain (V770-NP1-R), and the British an alum-precipitated cell-free filtrate of a Sterne strain culture. The US vaccine has been shown to induce high levels of antibody only to protective antigen, while the British vaccine induces lower levels of antibody to protective antigen but measurable antibodies against lethal factor and edema factor.[52,53] Neither vaccine has been examined in a human clinical efficacy trial, although a study using a vaccine similar to the current US product was carried out in at-risk mill workers in the northeastern United States. The vaccine had an overall efficacy rate against cutaneous anthrax of 92.5%, although it should be noted that the study was not sufficiently statistically powered to assess protection against inhalational anthrax. Thirty-five percent of the recipients reported some type of reaction to vaccination. The preponderance of these events were minor, with 0.7% of recipients reporting systemic and 2.4% experiencing significant local effects with the first dose, rising to 1.3% with systemic and 2.7% with significant local effects with subsequent doses.[54] Manufacturer labeling for the current Michigan Department of Public Health anthrax vaccine adsorbed (AVA) product cites a 30% rate of mild local reactions and a 4% rate of moderate local reactions with a second dose.[55] The current complex dosing schedule for the AVA vaccine, derived from the aforementioned trial in mill workers, consists of 0.5 mL administered subcutaneously at 0, 2, and 4 weeks, and 6, 12, and 18 months, followed by yearly boosters.

Animal studies examining the efficacy of available anthrax vaccines against aerosolized exposure have been performed. While some guinea pig studies question vaccine efficacy,[56,57] primate studies support its role. In recent work, rhesus monkeys immunized with 2 doses of the AVA vaccine were challenged with lethal doses of aerosolized *B anthracis* spores. All monkeys in the control group died 3 to 5 days after exposure, while the vaccinated monkeys were protected up to 2 years after immunization.[58] Another trial used the AVA vaccine in a 2-dose series with a slightly different dosing interval, and again found it to be protective in all rhesus monkeys exposed to lethal aero-

sol challenge.[59] Thus, available evidence suggests that 2 doses of the current AVA vaccine should be efficacious against an aerosol exposure to anthrax spores. In addition, a highly purified, minimally reactogenic, recombinant protective antigen vaccine has been investigated, using aluminum as well as other adjuvants. Other approaches include cloning the protective antigen gene into a variety of bacteria and viruses, and the development of mutant, avirulent strains of *B anthracis*.[60-63]

BIOLOGICAL WARFARE ASPECTS

Recent incidents, such as the use of sarin in the Tokyo subway system and the bombing of the World Trade Center in New York City and the Oklahoma City Federal Building, as well as concerns over the potential use of biological and chemical weapons during the Persian Gulf War, underscore the threat of biological warfare either on the battlefield or by terrorists. Anthrax has been the focus of much attention as a potential biological warfare agent for at least 6 decades. Modeling studies have shown the potential for use in an offensive capacity. Dispersal experiments with the simulant *Bacillus globigii* in the New York subway system in the 1960s suggested that release of a similar amount of *B anthracis* during rush hour would result in 10 000 deaths.[64] On a larger scale, the World Health Organization estimated that 50 kg of *B anthracis* released upwind of a population center of 500 000 would result in up to 95 000 fatalities, with an additional 125 000 persons incapacitated.[65] Both on the battlefield and in a terrorist strike, *B anthracis* has the attribute of being potentially undetectable until large numbers of seriously ill individuals present with characteristic signs and symptoms of inhalational anthrax.

Given these findings, efforts to prevent disease are of obvious importance. The US military's current M17 and M40 gas masks provide excellent protection against the 1- to 5-µm particulates needed for a successful aerosol attack. Assuming a correct fit, these masks would be highly effective if in use at the time of exposure. Some protection might also be afforded by various forms of shelter. The preexposure use of the current AVA anthrax vaccine, which is approved by the US Food and Drug Administration, appears to be an important adjunct. Results of primate studies also support the concept of postexposure antibiotic prophylaxis. Work by Friedlander et al[66] showed that 7 of 10 monkeys given penicillin, 8 of 9 given ciprofloxacin, 9 of 10 treated with doxycycline, and all 9 receiving doxycycline plus postexposure vaccination survived a lethal aerosol challenge, with all animals receiving antibiotics for 30 days following exposure. Earlier research suggested that short courses of prophylactic antibiotics delayed but did not prevent clinical disease.[67] Accordingly, in the event of documented exposure, prolonged prophylactic antibiotic use, as well as vaccination, would be mandatory. In the biological warfare setting, the differential diagnosis of inhalational anthrax would include plague and tularemia. Fluoroquinolones also have activity against these diseases, supporting the use of ciprofloxacin and perhaps other drugs of this class as either a preexposure or postexposure measure.

CONCLUSION

The inhalational form of anthrax remains a legitimate and perhaps growing military and terrorist threat in the current world situation. Knowledge of inhalational anthrax is necessary for public health officials, as well as the health care providers who would be called on to care for casualties. Important methods of prevention include properly fitting protective masks capable of filtering 1- to 5-µm particles, the use of preexposure and postexposure antibiotics, and the use of preexposure and postexposure vaccination. All these measures would be expected to provide a substantial degree of protection against aerosolized *B anthracis*; not all, however, are easily applicable to a civilian setting. Consequently, the morbidity and mortality of an attack might still be high.

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