Chapter 31

Anthrax Vaccine

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Anthrax, a zoonotic disease caused by *Bacillus anthracis*, has three forms: cutaneous, inhalational, and gastrointestinal. Mortality in untreated cutaneous cases is about 20%, and less than 1% if antibiotics are given. Inhalational anthrax is almost 100% fatal if untreated, and gastrointestinal cases have an untreated mortality rate of 25% to 75%. Meningitis may be a complication of any of the three forms. Natural cases primarily are associated with industrial, agricultural, or laboratory exposure. The natural disease is not a major public health problem in the world today, although occasional epidemics do occur. However, the intentional use of *B. anthracis* as a bioterrorist weapon in the fall of 2001 irrevocably altered our views of public health, not just for anthrax but also for many other infections.

Historically, anthrax is considered to have been the fifth and sixth plagues described in Exodus (circa 1491 BC). Hippocrates described the disease in approximately 300 BC. Europeans recorded epizootics and epidemics in the 16th century. Between 1750 and 1850, the disease in humans and animals was described in detail, and the organism was characterized.

In the 1870s, Koch cultured *B. anthracis* on artificial media and demonstrated definitively for the first time the microbial etiology of an infectious disease. In 1881, Pasteur attenuated the organism and conducted a successful field test of his vaccine for livestock. Greenfield performed similar work at the same time.[1] In the late 1800s and early 1900s, cases of cutaneous and inhalational industrial anthrax involved rag pickers in Germany and wool sorters in England.[2] The term *woolsorters' disease* referred to inhalational anthrax. Because of the large number of reported cases in England, Britons established a wool disinfection station in Liverpool.[3] All incoming wool and other animal fibers were disinfected using formaldehyde baths before being further processed. Subsequently, the number of cases of anthrax among these workers decreased significantly.

Cases of human anthrax have been reported from almost every country. However, the actual number of cases in the world is at best an estimate. In 1958, Glassman estimated the annual worldwide incidence at 20,000 to 100,000 cases.[4] In the 1980s and 1990s, the global total decreased to an estimated 2000 cases annually.

Industrial cases occurred primarily in European and North American countries, associated with the processing of animal materials, such as hair, wool, hides, and bones. Agricultural cases occur primarily in Asian and African countries and result from contact with diseased domestic animals or their products, such as hair, wool, hides, bones, and carcasses, including meat.

In the United States, the earliest reports of animal anthrax came in the early 1700s from what is now Louisiana. Sporadic animal cases were reported later from almost every state. Areas with more regularly reported cases are now called anthrax districts and primarily include the Great Plains states. Human anthrax was first reported from Kentucky in 1824. Human cases subsequently occurred throughout the United States, with the majority reported from industrialized states in the Northeast. However, as the textile industry moved to other parts of the country, human cases arose in the new locations.

Several unusual epidemics have been reported since the late 1970s. The largest epidemic in modern times occurred in Zimbabwe, with approximately 10,000 human cases reported between 1979 and 1985, including approximately 7000 cases occurring in 1979 and 1980.[5–7] Most of the affected people had cutaneous lesions, but some gastrointestinal cases also were reported. The source of infections was infected cattle.

Another unusual epidemic occurred in Sverdlovsk, Russia, in 1979. After an accidental release of spores from a military microbiology facility, at least 77 human cases of inhalational anthrax with at least 66 deaths occurred among people exposed to an aerosol containing *B. anthracis* organisms.[8,9] Some cases also occurred in sheep and cattle grazing up to 50 km downwind from the facility, possibly as a result of the same release, although natural anthrax outbreaks previously had been reported from the region. Iraq's admission to the United Nations that it produced weapons containing anthrax spores and was prepared to launch them during the 1991 Persian Gulf War confirmed fears of the potential use of *B. anthracis* as a biologic weapon.[10]

In late September 2001, a Florida man developed inhalational anthrax, the first case in the United States since 1976. He subsequently died.[11] Initially thought to be an isolated case, he was the first diagnosed case among 11 confirmed inhalational cases and 7 confirmed and 4 suspected cutaneous

cases of anthrax reported from Florida, New York, New Jersey, the District of Columbia, and Connecticut.[12–14] Exposure to contaminated mail was the confirmed or apparent source of infection in all patients.[12–15] Among cutaneous cases, lesions developed on the forearm, neck, chest, or fingers.[16] Of the 11 inhalational cases, the median age was 56 years (range 43 to 94 years). The average incubation from known exposure to symptoms was 4 days (range 4 to 6 days).[14]

The incidence of human anthrax in the developed world is extremely low. The only impetus for the development of an improved human vaccine is the threat of *B. anthracis* used as a biologic weapon. This horrendous possibility was unfortunately given credence by the 1979 Sverdlovsk incident and the 1991 Iraqi experience. These events prompted the U.S. Department of Defense to begin anthrax vaccinations for some members of the Armed Forces. The 2001 bioterrorist-related anthrax outbreaks in the eastern United States confirmed our fears and heightened interest in the effort to develop new vaccines. The specter of anthrax used as a bioterrorist weapon against civilian populations on a larger scale than that yet experienced poses possible catastrophic consequences.[17] Given that spores can persist in experimentally infected animals after treatment with antibiotics for more than 30 days,[18–21] the major efforts in public health management of such an event focus on early diagnosis and postexposure prophylaxis with both antibiotics and vaccination.

Background

Clinical Description

There are three primary forms of anthrax: cutaneous, inhalational, and gastrointestinal.[22,23] Secondary meningitis can occur with all three forms of anthrax. Rarely, a case of anthrax meningitis has been reported in which the primary site was not identified. In the United States, approximately 95% of reported cases have been cutaneous and 5% inhalational. There have been no confirmed gastrointestinal cases in the United States.

Cutaneous Anthrax

The incubation period for cutaneous anthrax is 1 to 7 days (usually 2 to 5 days). The lesion is first noted as a small, pruritic papule. Within several days, the papule develops into a vesicle that may be 1 to 2 cm in diameter. Occasionally, the initial papule is surrounded by a ring of vesicles, which then coalesce to form a large vesicle. The vesicular fluid is clear or serous colored and contains numerous *B. anthracis* organisms and a paucity of leukocytes. Nonpitting edema and erythema may develop around the lesion. Pain is not present unless there is secondary infection. The vesicle may enlarge to 2 to 3 cm in diameter, sometimes becoming hemorrhagic. Systemic symptoms are usually mild and can include malaise and low-grade fever. There may be regional lymphangitis and lymphadenopathy. Approximately 5 to 7 days after the onset of disease, the vesicle ruptures, revealing a straight-edged, depressed ulcer crater that develops a typical black eschar. Over a period of 2 to 3 weeks, the eschar loosens and falls off, most often without scar formation. The evolution of the lesion is not affected by antibiotic treatment.

The lesion most often occurs on an exposed part of the body, such as the face, neck, or arm. Large, irregularly shaped cutaneous lesions have been seen in some industrial cases that developed when many organisms were rubbed into the skin. Occasionally, a lesion involving the ocular area is more extensive. The orbit may become involved, with subsequent damage to the lids and ductal system.

More severe cutaneous involvement occasionally occurs that is referred to as *malignant edema*, in which multiple bullae surround the site of the initial lesion and extensive local edema, induration, and toxemia are present. At times the edema may be massive, extending from a primary lesion on the neck to the groin.

Rarely, multiple cutaneous lesions have occurred that probably represent multiple inoculations of spores through the skin. Reinfections have been reported very rarely but not confirmed.

Inhalational Anthrax

One to 5 days after inhaling an infectious dose of *B. anthracis* organisms, nonspecific symptoms develop that include malaise, fatigue, myalgia, slight temperature elevation, and minimal nonproductive cough. Symptoms similar to an upper respiratory infection are characteristically absent. There may be a feeling of precordial oppression. Auscultation of the chest may reveal rhonchi. A slight improvement may occur within 2 to 4 days, but then severe respiratory distress develops suddenly, including dyspnea, cyanosis, stridor, and profuse diaphoresis. In some cases, subcutaneous edema of the neck and chest may be present. Physical examination reveals a patient with toxic symptoms who has an elevated pulse, respiratory rate, and temperature. Physical exam may reveal signs of a pleural effusion. Widening of the mediastinum on radiographic examination of the chest is frequently seen, as are pleural effusions. The leukocyte count may be elevated moderately. Shock may develop, and death usually occurs within 24 hours of the onset of the acute phase. Death likely is caused by lymphatic/vascular obstruction in the mediastinum, with pulmonary hemorrhage and edema associated with large pleural effusions and toxicity. The clinical courses of five patients in a goat hair epidemic are shown in Figure 31–1.[24]

The patients treated in 2001 frequently reported chills, prolonged fatigue, nausea or vomiting, and chest discomfort.[14] None had an initially normal chest radiograph[13] (Table 31–1). They frequently manifested paratracheal fullness, hilar fullness, and pleural effusions or infiltrates or both; in some patients these initial findings were subtle. Among all eight patients who had not received antibiotics before diagnosis, *B. anthracis* grew in blood cultures drawn at initial examination. Six of the 11 (55%) survived with aggressive supportive care and multidrug antibiotic regimens.[13,14] All four individuals who exhibited fulminant signs of illness, with severe respiratory distress or hypotension or meningitis when they presented, died despite receiving antibiotics active against *B. anthracis*.[13]

Anthrax meningitis has been reported to occur in approximately 50% of inhalational anthrax cases, but it can develop after bacteremia secondary to the other forms and, very rarely, without an obvious primary source. Clinically, it resembles other meningitides, although it is frequently hemorrhagic.

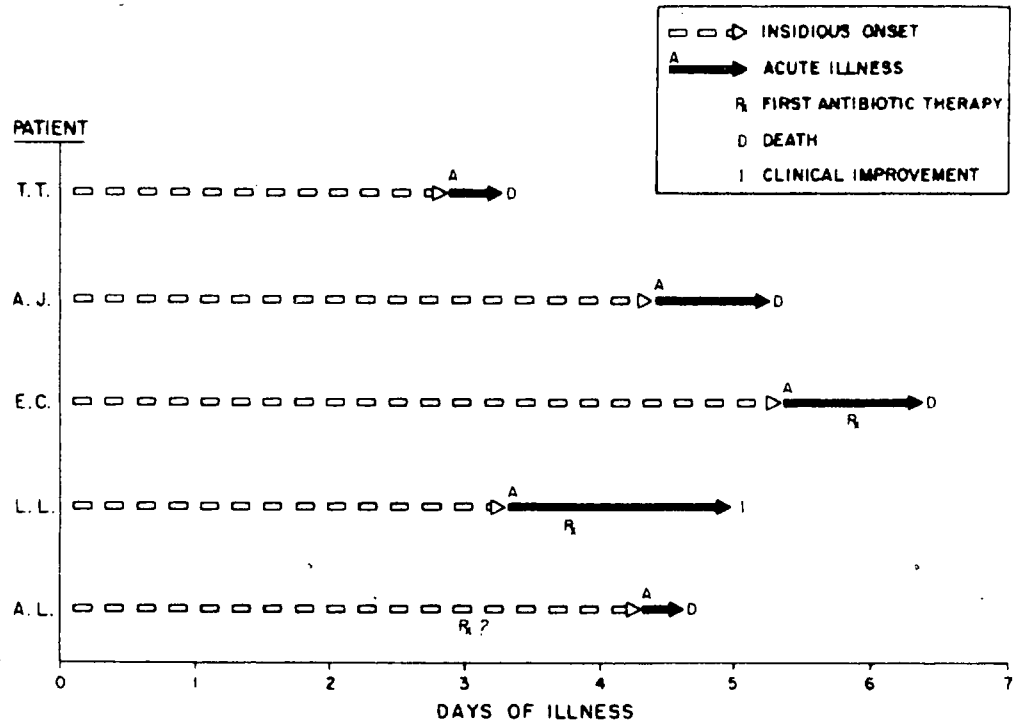

FIGURE 31–1 ▪ Diagrammatic representation of the two stages of inhalation anthrax exemplified by the Manchester patients: insidious onset (→) and acute toxemia (A→). The occurrence of first antibiotic treatment (Rx) and death (D) or improvement (I) is shown in temporal relationship to these stages.[24]

Gastrointestinal Anthrax

Symptoms of gastrointestinal anthrax develop 2 to 5 days after the ingestion of contaminated meat. The initial symptoms of disease consist of nausea, vomiting, anorexia, and fever followed by abdominal pain and diarrhea, which may be bloody. Hematemesis, possibly severe, may develop. In some cases, the presentation is that of an acute abdomen and has prompted surgical exploration of the abdomen. Physical examination reveals an elevated temperature, pulse, and respiratory rate. Sepsis with toxemia, shock, and death may develop.

Oral-oropharyngeal anthrax occurs when ingested organisms gain entrance to the subcutaneous tissues through the oral or oropharyngeal tissue. In these cases, local ulcers, fever, anorexia, cervical or submandibular lymphadenopathy, or edema may develop.

Bacteriology

B. anthracis, the causative agent of anthrax, is a large, gram-positive, spore-forming, nonmotile bacillus (1.0 to 1.5 × 3 to 10 μm). The organism grows readily on sheep blood agar aerobically and is nonhemolytic under these conditions. The colonies are large, rough, and gray-white, with irregular, tapered, curving outgrowths that cause the typical "Medusa head" appearance. A loop drawn up through a colony makes the disturbed part of the colony stand upright like whipped egg white. In the presence of high concentrations of carbon dioxide, the organisms form antiphagocytic capsules, and colonies are smooth and mucoid. In tissue, the bacteria are encapsulated and appear singly or in chains of two or three bacilli. Bacterial identification is confirmed by the production of toxin antigen; lysis by a specific gamma bacteriophage; the presence of a capsule and cell wall polysaccharide, as determined by fluorescent antibody testing; and virulence for mice and guinea pigs. Polymerase chain reaction tests for toxin and capsule genes are also confirmatory. Genetic analyses of different isolates reveal that *B. anthracis* is one of the most monomorphic, homogeneous bacterial species known.[25,26]

The spores are quite resistant to environmental extremes, and may survive for decades in certain soil conditions. Viable spores were reported to persist for weeks to months within the lungs of rhesus monkeys after inhalation, at which time they are still capable of germinating and causing fatal infection.[18–21]

TABLE 31–1 ▪ Initial Clinical Findings in 10 Patients with Bioterrorism-Related Inhalational Anthrax, October through November, 2001

Chest radiography findings	
Any abnormality	10/10
Mediastinal widening	7/10
Infiltrates/consolidation	7/10
Pleural effusion	8/10
Chest computed tomography findings	
Any abnormality	8/8

Pathogenesis

The known virulence determinants of *B. anthracis* important in pathogenesis are the capsule and two protein exotoxins. The importance of the capsule was appreciated early in this century when Bail demonstrated that organisms that lost the ability to produce capsule were avirulent.[27]

Extensive studies by Sterne[28] and others in the 1930s expanded this idea and further showed that such nonencapsulated strains could induce immunity to anthrax, thus demonstrating that the capsule is not necessary to induce protective immunity. The strains developed by Sterne have proved remarkably effective as live vaccines for domesticated animals and are used worldwide.[29]

As is true for many bacterial virulence factors, the genes encoding the anthrax capsule are carried on an extrachromosomal 96-kilobase plasmid (pX02).[30,31] This discovery allowed more definitive confirmation that the capsule is necessary for virulence. Anthrax strains lacking the capsule plasmid failed to produce capsule and were attenuated.[32] The capsule, a protein composed of poly-D-glutamic acid, enhances virulence by making the organism resistant to phagocytosis and also may protect the bacilli from lysis by cationic proteins in serum.[33] Although the capsule is a necessary virulence factor, it is not an effective immunogen in most experimental animals.

A role for toxins in anthrax pathogenesis was suspected from the earliest studies of Koch,[34] but it was not firmly established until 1954, when Smith and Keppie demonstrated that sterile plasma from experimentally infected guinea pigs was lethal after being injected into other animals.[35] Evans and Shoesmith showed that *B. anthracis* culture filtrates produce edema after injection into the skin of rabbits.[36] Much work was done in the 1950s and 1960s to study the role of toxins in disease and immunity.[37,38] Although since the mid-1980s there have been great advances in our understanding of the molecular biology of the toxins,[39] their exact role in pathogenesis remains less well defined. Anthrax has been characterized as being due to a large bacterium that produces a feeble toxin.[40] Although it is clear that anthrax is an invasive disease and that the lethal toxin, when given intravenously, is relatively impotent compared with other bacterial toxins, both the lethal and edema toxins are thought to be important in the establishment of disease by impairing host defenses.

The anthrax toxins, like many bacterial toxins (e.g., diphtheria, tetanus, botulinum), possess a binding domain by which they bind to target cell receptors and an active domain that is responsible for the biochemical and usually enzymatic activity of the toxin. The anthrax toxins are unusual in that the binding and active domains are present on two distinct proteins, and the two toxins share the same binding protein. This binding protein is called protective antigen (PA). PA combined with a second protein called lethal factor (LF) constitutes the anthrax lethal toxin, which is lethal when injected into experimental animals.[41,42] The same PA combined with a third protein, edema factor (EF), constitutes the edema toxin, which causes edema when injected into experimental animals.[41,42] The edema toxin is undoubtedly responsible for the massive edema that may be present in cases of anthrax, especially inhalational anthrax. The 89-kDa EF is a calmodulin-dependent adenylate cyclase that raises intracellular cyclic AMP levels.[43] The 85-kDa LF has been shown to be a zinc metalloprotease that inactivates mitogen-activated protein kinase kinases,[44,45] although the exact cellular target responsible for its biologic effect remains unknown. Consistent with this model, each of the individual proteins alone lacks biologic activity.

The crystal structures of PA,[46] LF,[47] and EF[48] have all been determined. The current model based on in vitro cell culture studies suggests that the PA molecule first binds to a host cell anthrax toxin receptor.[49] The PA molecule then is cleaved by a cell-surface protease, releasing a 20-kDa amino-terminal fragment. The cell-bound 63-kDa carboxy-terminal fragment heptamerizes and creates a second binding domain to which either or both of the active proteins (i.e., the lethal or edema factor) binds. The complex then enters the cell through endocytosis and exerts its toxic effect within the cytosol.

The genes for the toxin proteins are carried on a second 182-kilobase plasmid (pX01).[50] The pathogenic role of the toxins was demonstrated clearly when strains deleted from the plasmid coding for the toxin genes but still encapsulated were shown to be attenuated.[32,50] Of historical significance, it appears that the veterinary vaccine strains produced by Pasteur by passage at high temperature do not contain the plasmid for the toxin genes.[50] This characteristic explains the lack of virulence of these vaccines. Further work has shown that deleting the PA gene alone eliminates the organism's virulence,[51] thus confirming the central role of PA in the activity of the two toxins as well as their role in virulence.

Early studies showed that crude toxin preparations or combinations of edema and lethal toxins inhibited neutrophil killing,[52] chemotaxis,[53] or phagocytosis.[33] More recent work has shown that the edema toxin inhibits neutrophil phagocytosis[54] and priming of the respiratory burst of neutrophils.[55] Evidence exists that, at low concentrations, lethal and edema toxins may block the production of proinflammatory cytokines,[56,57] and so interfere with the early protective inflammatory response. Some evidence also suggests that the lethal toxin acts on macrophages to release the cytokines interleukin-1β and tumor necrosis factor-α,[23,58] whereas at higher concentrations, it is specifically cytolytic for these cells.[59] In terms of pathogenesis, the greater importance of lethal toxin versus edema toxin was demonstrated with a mouse model in which an anthrax strain containing the lethal toxin alone retained some virulence, whereas a strain containing only the edema toxin was avirulent when compared with the parent strain containing both toxins.[60]

Infection begins when the spore is introduced through the skin or mucosa. At the local site, the spore germinates into the vegetative bacillus with production of the antiphagocytic capsule. The edema and lethal toxins produced by the organism impair leukocyte function and contribute to the distinctive findings of tissue necrosis, edema, and relative absence of leukocytes. If not contained, the bacilli spread to the draining regional lymph nodes, thereby leading to the further production of toxins and the induction of the typical hemorrhagic, edematous, and necrotic lymphadenitis. From the lymph nodes, the bacteria multiply further and enter the blood stream to produce a systemic infection.

In inhalational anthrax, spores are ingested by alveolar macrophages and are transported to the tracheobronchial and mediastinal lymph nodes, where they germinate.[61] Local production of toxins by extracellular bacilli leads to the massive hemorrhagic, edematous, and necrotic lymphadenitis and mediastinitis that is so characteristic of this form of the disease. The bacilli then spread through the blood, causing septicemia and, at times, hemorrhagic meningitis. Late in the disease, toxin is present in the blood at high concentrations, with the lethal toxin occurring as a complex of PA and LF

site of action and the role of lethal toxin in the mechanism of death from infection remain obscure, but the uncontrolled release of cytokines and other possible mediators from macrophages may be involved. Death is due to respiratory failure with overwhelming bacteremia that is often associated with meningitis and subarachnoid hemorrhage.

Diagnosis

Diagnosis of cutaneous anthrax should be considered after the appearance of a painless, pruritic papule that develops into a vesicle, revealing a black eschar at the base of a shallow ulcer. Examination by Gram's stain or culture of the vesicular fluid should confirm the diagnosis, but prior antibiotic therapy quickly renders the infected site culture negative. Biopsy at the lesion edge, examined by Gram's stain, immunohistology, and polymerase chain reaction, may be useful in people treated with antibiotics. In addition, there should be a history of exposure to materials that have been contaminated with *B. anthracis*.

The diagnosis of inhalational anthrax is difficult, but it should be suspected in cases with a history of exposure to an aerosol that contains *B. anthracis*, followed by an initial phase during which the symptoms of inhalational anthrax are nonspecific, as described previously. Once the acute stage has developed, a widened mediastinum seen on a chest radiograph, often with pleural effusions, should suggest the diagnosis. In untreated cases, culture of blood and pleural effusions will readily establish the diagnosis. In cases previously treated with antibiotics, polymerase chain reaction of blood and pleural fluid, as well as immunohistochemical examination of pleural fluid or transbronchial biopsy specimens, are of value, as demonstrated in the recent outbreak.[13,14,16] Because primary pneumonia is not usually a feature of inhalational anthrax, sputum examinations do not aid diagnosis. The radiographic differential diagnosis should include histoplasmosis, sarcoidosis, tuberculosis, and lymphoma. A computed tomography scan of the chest may be helpful to detect mediastinal hemorrhagic lymphadenopathy and edema, peribronchial thickening, and pleural effusions.

Gastrointestinal anthrax is difficult to diagnose because of its rarity and similarity to other more common severe gastrointestinal diseases. An epidemiologic history of ingesting contaminated meat, particularly in association with other similar cases, should suggest the diagnosis. Microbiologic cultures are not helpful in confirming the diagnosis unless bacteremia is present. The diagnosis of oral-oropharyngeal anthrax can be made from the clinical and physical findings. Adequate data are not available to assess the value of bacteriologic cultures in confirming the diagnosis.

Treatment and Prevention with Antibiotics

Mild cases of cutaneous anthrax may be treated effectively orally with a penicillin, a tetracycline, or another antibiotic, depending on antimicrobial resistance. If spreading infection or prominent systemic symptoms are present, then high-dose parenteral therapy should be given as for inhalational anthrax until there is a clinical response. Effective therapy reduces the edema and systemic symptoms but does not change the evolution of the skin lesion itself.

Treatment of inhalational or gastrointestinal anthrax requires high-dose intravenous therapy with two or more antibiotics, to include a fluoroquinolone or doxycycline.[13,14,21,63–66] Limited animal data suggest that the addition of an aminoglycoside to penicillin treatment would provide additional benefit. Regimens should be altered based on susceptibility testing and clinical status. The successful treatment of 6 of the 11 inhalational cases in the 2001 bioterrorist attacks suggests that, with rapid treatment with effective antibiotics and modern supportive care, including aggressive management of pleural effusions, mortality is similar to that of other causes of sepsis.

Prophylactic treatment to prevent anthrax after exposure to an infectious spore aerosol should include oral antibiotics for 30 to 60 days or more, depending on individual circumstances (e.g., extent of exposure, vaccination status).[66–68] The Food and Drug Administration confirms the evidence for safety and efficacy of ciprofloxacin, doxycycline, and penicillin G procaine for this indication,[65] with amoxicillin recommended for children and pregnant or lactating women, depending on microbial resistance.[14,63,64,66,69] Pre- or postexposure vaccination may enable shorter courses of antibiotics.[66,70] Postexposure vaccination alone would not be expected to be effective.[21]

Epidemiology

Several theories explain the ecology of soil infected with *B. anthracis*. One theory suggests that *B. anthracis* spores can persist for many years in some types of soil under certain conditions. These conditions are a soil rich in nitrogen and organic material and with adequate calcium, a pH greater than 6.0, and an ambient temperature greater than 15.5°C. It remains unclear whether there are cycles of germination and replication within the soil or if amplification within mammalian hosts serves to maintain the population in the soil between outbreaks in animals.

Animal anthrax results from animals ingesting *B. anthracis* spores, either from eating contaminated feed or while grazing on pastures. Soil becomes contaminated from contaminated fertilizer or contaminated feed spread on the ground or from diseased animals that contaminate the soil with their secretions before or after death.[71]

The number of reported human anthrax cases in the United States has declined steadily since adequate surveillance data have been available. Between 1916 and 1925, the annual average number of cases was 127; between 1948 and 1957, 44 cases; between 1978 and 1987, 0.9 case; and between 1988 and 2000, 0.25 case. Of the 235 human cases reported from 1955 to 2000, 20 were fatal (Fig. 31–2).[72,73] Among these cases, 224 had cutaneous lesions (118 on an arm, 65 on the head or neck, 11 on the trunk, 8 on a leg, and 22 at an unknown site) and 11 were inhalational cases.

The traditional classification of cases is related to the source of infection, that is, whether it is acquired in an industrial, an agricultural, or a laboratory setting. The basic epidemiologic principles are the same in developing and developed countries. Agricultural anthrax is a more significant problem in developing countries, and industrial anthrax occurs more commonly in developed countries. Industrial anthrax results from the exposure of susceptible

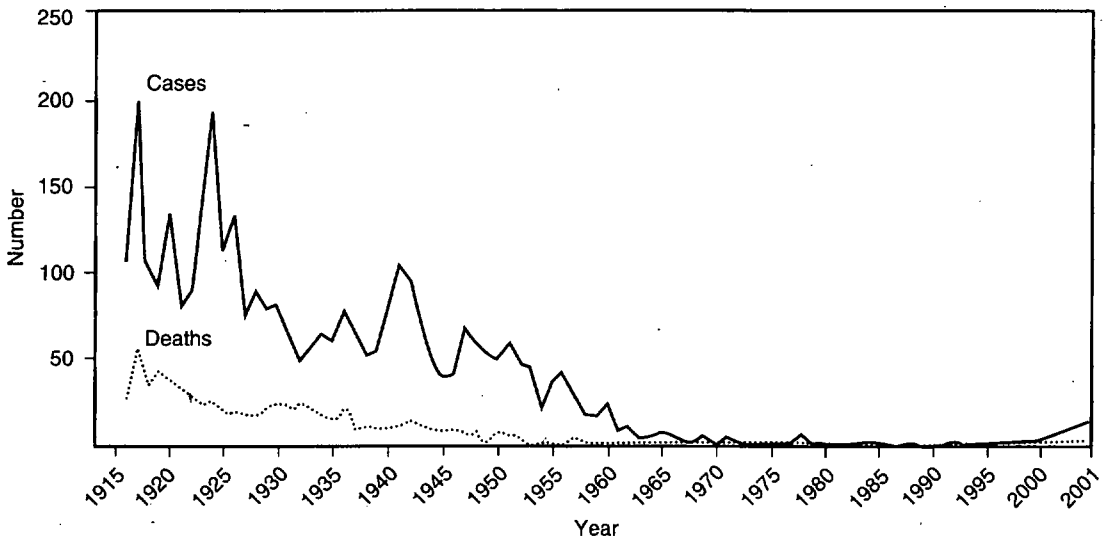

FIGURE 31–2 ▪ Number of cases of anthrax and deaths caused by anthrax in humans, United States, 1916 to 2001. (Data from the Centers for Disease Control and Prevention. [72,73])

individuals to contaminated animal products that include wool, goat hair, hides, or bones. These materials come from animals that either were infected with *B. anthracis* before death or are contaminated after death (e.g., from contaminated soil with which the carcass or animal products came into contact). The wool and hair from infected animals may be clipped from live animals or pulled from carcasses. A hide may be obtained from an animal that has died of anthrax. Bones can be collected from grazing areas on which animals die or from rendering plants that may handle carcasses of animals that have died from anthrax.

Wool and goat hair are processed into yarn that is used in the textile and carpet industries or in the preparation of other cloth-like materials. Hides are processed into leather goods. Bones are used in preparing bone meal, gelatin, and fertilizer. In industrial cases, cutaneous anthrax results from spores that gain entrance through the skin by entering preexisting wounds or by being rubbed through the skin or on a hair fiber that may penetrate the skin. At times, the processing of goat hair and wool creates infectious aerosols that may result in inhalational anthrax when inhaled. A rendering plant is another source of potential infection.

Cases associated with agricultural settings result from contact with diseased animals or with the products of animals that have died of anthrax. Affected individuals are primarily agricultural workers, veterinarians, or individuals who kill and butcher infected animals or butcher the carcasses of animals that have died of anthrax. This contact results in cutaneous anthrax or, if the infected meat is ingested, gastrointestinal or oral-oropharyngeal anthrax.

Laboratory-associated cases of anthrax are rare. These are essentially all cutaneous, although a few inhalational cases have occurred. Rarely, cases have been reported after contact with contaminated clothing, such as woolen coats or pilots' leather helmets. Table 31–2 presents the sources of infection of the 257 cases reported in the United States from 1955 to 2001. The two vaccine-associated cases of agricultural anthrax resulted from the inadvertent injection of animal vaccine into the hand of the vaccinator.

Exposures related to bioterrorist events represent a new category. The anthrax terror attacks of fall 2001 resulted in 11 confirmed inhalational cases and 7 confirmed and 4 suspected cutaneous cases of anthrax reported from Florida, New York, New Jersey, the District of Columbia, and Connecticut.[12–14] Exposure to contaminated mail was the confirmed or apparent source of infection in all patients.[14,15] More than 32,000 people received short courses of prophylactic antibiotics while potential exposures were evaluated,[67] and among these more than 10,000 people received 60 days or more of antibiotics with or without postexposure vaccination as prophylaxis.[68,74] Exposures may have resulted from opening contaminated letters or packages, from working in buildings with high-speed automated mail-sorting machines, or through contact

TABLE 31–2 ▪ Sources of Infection in 257 Cases of Human Anthrax in the United States, 1955 to 2001

Industrial (No.)	Agricultural (No.)	Terrorism (No.)
Goat hair (113)	Animal (42)	Inhalational
Wool (34)	Vaccine (2)	Working in mail-processing facility (7)
Goat skin (16)	Unknown (8)	Receiving mail (confirmed or presumptive) (4)
Meat (3)	**Total**: 52	Cutaneous
Bone (4)		Working in mail-processing facility (7)
Unknown (13)		Receiving mail (confirmed or presumptive) (4)
Total: 183		**Total**: 22 (18 confirmed and 4 suspected)

with cross-contaminated pieces of mail or environments contaminated with spores.

Passive Immunization

In the era before antibiotics, animal antisera were common therapeutic products.[75] One of the first was anthrax antiserum, developed in France by Marchoux and in Italy by Sclavo in 1895.[76,77] Although it was used initially for prophylaxis and treatment of anthrax among livestock, Sclavo later used his product to treat human disease, either cutaneous or septicemic. He reported 10 deaths among 164 treated patients (6% mortality, compared to the Italian case-fatality rate of 24%). Sclavo injected 30 to 40 mL of antiserum subcutaneously, repeated 24 hours later. In severe cases, he also injected 10 mL or more intravenously.

Between the 1910s and 1940s, clinicians in Europe and the Americas treated patients with anthrax antiserum using 25 to 300 mL daily for 5 days, sometimes in combination with arsenicals.[75–86] One patient with severe cutaneous anthrax recovered after receiving 2265 mL of antiserum.[87] No controlled studies were performed to demonstrate efficacy. Anthrax antiserum for therapy of cutaneous anthrax was superseded by therapy with sulfanilamide, followed by penicillin and other antibiotics.[85,88]

Equine anthrax antiserum produced by live-spore vaccination has been licensed in China,[89] the Soviet Union, and later Russia for decades, and its use continues, although the magnitude or frequency of use is unclear. In recent years, the Lanzhou Institute of Biological Products in China developed a lyophilized anti-anthrax $F(ab)_2$ formulation of equine immunoglobulin G (IgG) fragments, for human use by intracutaneous, intramuscular, or intravenous administration, but it is little used (Dong Shulin, personal communication, 2002).

Experimental evidence indicates that passive immunization with equine antibody produced against attenuated Sterne veterinary vaccine strains or against crude toxins prevents disease in animals when given before or shortly after spore challenge.[18,90] Rhesus monkeys could be protected with one or two doses of equine anti-anthrax spore hyperimmune serum when begun 1 day after low-dose aerosol challenge. Forty-five percent of immune serum–treated animals survived, compared to 10% of controls.

More recent studies by Little et al. showed efficacy of anti-PA antiserum prophylaxis against an intramuscular challenge in animals.[91] The anti-PA polyclonal antibody protected against death and anti-PA monoclonal antibody significantly delayed mortality. Reuveny and colleagues similarly found in passive immunization studies of guinea pigs that polyclonal anti-PA antisera conferred protection against an intradermal challenge dose of 40 median lethal doses (LD_{50}).[92]

Kobiler and colleagues challenged guinea pigs intranasally with a 25 LD_{50} dose of spores.[93] The animals were then treated with anti-PA, anti-LF, or anti–Sterne vaccine antibodies. Intraperitoneal administration of rabbit anti-PA serum 24 hours after infection protected 90% of infected animals, with lesser efficacy seen with anti-Sterne and anti-LF antibodies. Beedham and colleagues demonstrated that mice could be protected against challenge with a vaccine strain using serum but not spleen lymphocytes from PA-vaccinated animals, supporting the long-standing evidence that antibody is the major mechanism of vaccine-induced immunity.[94]

Although the importance of anthrax toxins in pathogenesis suggests that antiserum may play a role in treatment, modern western interest in such products for human use was not rekindled until the anthrax bioterrorism attacks in the fall of 2001.[95] The need for therapeutic tools other than antibiotics may be especially great in the case of antibiotic-resistant strains of *B. anthracis*, although there remains no definitive evidence to date of efficacy in humans.

Active Immunization

History of Vaccine Development

Although there is great historical interest in Pasteur's development of the first effective live bacterial vaccine, and live, attenuated veterinary vaccines are still used, human vaccines against anthrax consist of proteins purified from anthrax cultures, except as indicated in the following discussion. Early human anthrax vaccines (presumably live) were used in the 1910s but found little favor.[77] Sterne developed live, attenuated strains in the 1930s, which led to worldwide use for domesticated animals.[29] Russian investigators developed similar vaccines for both animal and human use. In 1946, Gladstone identified the PA component of cultures of *B. anthracis*.[96] Belton and Strange increased the yields of PA to allow large-scale production,[90] leading to the current British vaccine. Wright and colleagues used similar techniques to develop the precursors to the American vaccine.[97–99]

There has been confusion in the older literature over the use of the term *protective antigen*. Before the identification of the anthrax toxins, this term was applied to uncharacterized material derived from sterile extracts of experimental anthrax lesions[100,101] or from crude culture supernatants,[96] which were effective immunogens in experimental animals. *Protective antigen* is the term also applied to one of the toxin proteins, which is the plasmid-encoded binding component of the anthrax toxins described previously. It has become clear that these terms apply to the same protein. The major effective immunogen in culture supernatants is the PA component of the toxins, although smaller amounts of LF and EF may be present; their contribution to protective immunity has remained controversial.[37] In older studies, EF enhanced the protective efficacy of PA in some experimental animals.[102,103] The results of these studies are difficult to interpret because the preparations used may not have been pure and free from cross-contamination. Studies using the PA gene cloned into *B. subtilis* demonstrated conclusively that PA alone, in the absence of EF, LF, or other *B. anthracis* proteins, protects animals against experimental infection.[104] Although other experiments have shown that purer preparations of PA, free of immunologically detectable LF or EF,[105] or recombinant PA,[106] can protect experimental animals, it remains unknown whether adding EF or LF enhances the vaccine efficacy of PA.

Description of Vaccines

The human anthrax vaccine licensed in the United States, Anthrax Vaccine Adsorbed (AVA), is produced by the

BioPort Corporation (Lansing, MI) from sterile filtrates of microaerophilic cultures of an attenuated, unencapsulated nonproteolytic strain (V770-NP1-R) of *B. anthracis*. The cell-free culture filtrate, thought to contain predominantly PA, is adsorbed to aluminum hydroxide, and the final product contains no more than 2.4 mg of aluminum hydroxide per 0.5-mL dose. Formaldehyde, in a final concentration of no more than 0.02%, and 0.0025% benzethonium chloride are present as preservatives. Current product-content standards require 5 to 20 μg/mL of total protein, of which at least 35% is the 83-kDa PA protein, measured by densitometric analysis on sodium dodecyl sulfate–polyacrylamide gel electrophoresis after pooling 12 sublots.[107] It is unknown whether the PA is biologically active.

Some lots produced in Lansing in the 1980s appeared to contain small amounts of LF and lesser amounts of EF, as determined by induction of antibody responses in animal recipients,[32,105,108,109] although this has not been reported in the limited observations in human vaccinees.[109] Analysis found no detectable EF by Western blotting. Enzyme-linked immunosorbent assay (ELISA) studies found LF to be present in the range of 10 to 30 ng/mL of fermentation filtrate before adsorption.[107] Analysis by mouse macrophage cytotoxicity assay suggested that LF is present in a biologically inactive form.[107] Although it is clear that PA by itself is an effective immunogen, it remains unresolved whether the small amounts of LF or EF that may be present in some lots of the vaccine contribute to the vaccine's protective efficacy.

Potency testing of the BioPort vaccine is performed by assessing biologic activity after parenterally challenging guinea pigs. The vaccine is stored at 2° to 8°C. The recommended schedule for vaccination is 0.5 mL given subcutaneously at 0, 2, and 4 weeks, followed by 0.5-mL boosters at 6, 12, and 18 months. Studies of immunogenicity with intramuscular administration and fewer doses are underway. With continued exposure, additional yearly boosters are recommended. The vaccine is stable for 3 years after a successful potency test.

Anthrax Vaccine Precipitated, a similar vaccine from the Centre for Applied Microbiological Research (Porton Down, Salisbury, Wiltshire) was developed in the United Kingdom, first administered to humans in the early 1950s, and licensed in 1979.[110–114] This vaccine is made by precipitating the sterile cell-free culture filtrate of a derivative of the attenuated, noncapsulating Sterne strain $34F_2$ with aluminum potassium sulfate.[113] LF and EF are present in this vaccine at levels higher than believed to be found in lots of the U.S. vaccine from the 1980s.[109,115] The vaccine contains thimerosal as a preservative. The British vaccine is administered intramuscularly in a regimen of three 0.5-mL doses at 0, 3, and 6 weeks, with a booster dose 6 months after the third dose. Subsequent booster doses are given annually.[1]

A vaccine consisting of a suspension of live spores, named STI-1 for the Sanitary-Technical Institute, has been used for humans in the Soviet Union and its subsequent independent republics since 1953.[71,116] This strain, similar to the Sterne strain used in veterinary vaccines, is unencapsulated.[116] Although this vaccine has a reputation for causing substantial side effects, its developers assert that it is reasonably well tolerated and shows some degree of protective efficacy.[116–118] This vaccine, manufactured by the Tblisi Scientific Research Institute of Vaccines & Serums (Tblisi, Georgia), the Institute of Microbiology (Kirov [Viatka], Russian Federation), and perhaps other locations, is given by scarification through a 10- to 20-μL drop of vaccine containing 1.3 to 4×10^8 spores subcutaneously.[1,71,114,116,118–121] The initial dose is followed by a second dose 21 days later, with yearly boosters.

Another live spore human vaccine given by scarification has been manufactured by the Lanzhou Institute of Biological Products (Lanzhou, Gansu, People's Republic of China) since the 1960s, based on avirulent strain A16R.[71] A single dose contains 1.6 to 2.4×10^8 colony-forming units. A single booster dose is given 6 to 12 months after the first vaccination (Dong Shulin, personal communication, 2002).

Immunogenicity of Vaccine

The results of two studies indicated that immunization with the licensed U.S. vaccine induced an immune response (as measured by indirect hemagglutination) to PA in 83% of vaccinees 2 weeks after the first three doses,[122] and in 91% of those tested after receiving two or more doses.[123] The titers fell over time, but 100% of vaccinees responded with an anamnestic response to the annual booster dose. This hemagglutination assay correlated with results obtained by using an ELISA against PA,[124] which is the current test of choice. Analysis using a more sensitive ELISA against PA demonstrated that seroconversion occurs in 96% to 100% of vaccinees after the second dose.[125]

Using a more sensitive validated ELISA assay, Pittman found that one dose of Anthrax Vaccine Adsorbed evoked detectable anti-PA IgG antibodies in 60% to 84% of vaccinees.[126] After two doses, 95% to 100% of vaccinees developed anti-PA antibodies. The kinetics of anti-PA IgG response by this ELISA appear in Figure 31–3.[126] Prolonging the interval between the first two doses by a few weeks beyond the licensed 2-week interval increased antibody responses.[125,127] More extended intervals did not impair booster responses among Persian Gulf War troops given anthrax and botulinum vaccines after gaps of 18 to 24 months.[128] A preliminary study comparing subcutaneous and intramuscular administration of anthrax vaccine revealed higher titers to PA when a four week interval between the first two doses was compared to two weeks, and better tolerance of the vaccine by the IM route.[125] If confirmed by a larger study now in course, both the route of vaccination and the schedule of anthrax vaccine are likely to be improved.

Cellular Responses

Soviet scientists developed a skin-test antigenic reagent known as anthraxin in 1957, derived from the edematous fluid of infected animals given an unencapsulated *B. anthracis* strain.[121] Licensed in 1962, the skin test product is an autoclaved liquid composed of an undefined heat-stable polysaccharide-protein–nucleic acid complex without anthrax capsular or toxigenic material.[121,129] A positive skin test after 0.1 mL intradermal injection is defined as erythema ≥8 mm with local induration persisting for 48 hours.[121] Anthraxin demonstrated utility in identifying cases of anthrax[116,130] and identifying STI-1 vaccine–induced immunity in guinea pigs, sheep, and humans.[116] Experimental data show that guinea pigs vaccinated against anthrax that developed a positive anthraxin skin test were immune to a subsequent parenteral challenge.[121] Positive and negative predictive values of individual test results have not been published. There is limited

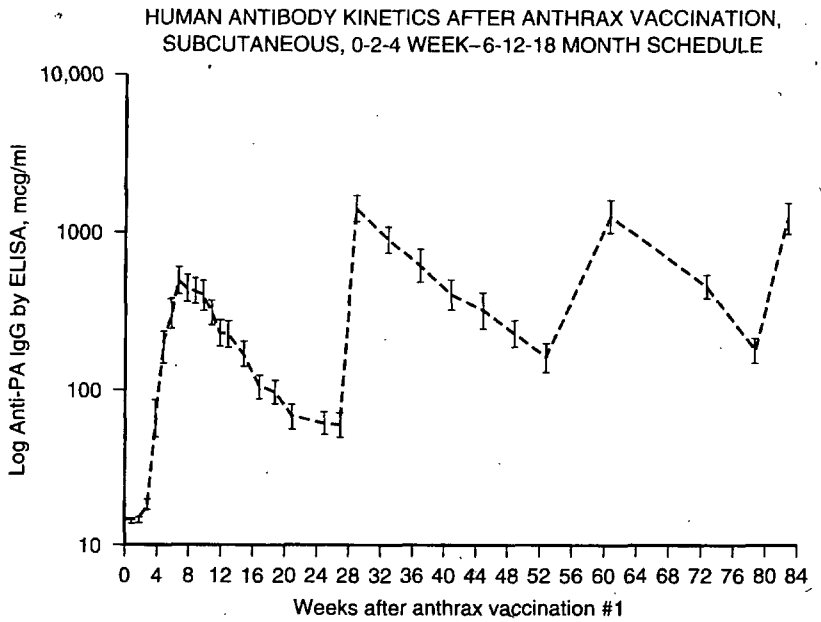

FIGURE 31–3 ▪ Anti-PA IgG antibody kinetics using the Food and Drug Administration–licensed dosing schedule. (Data from Pittman PR. Comparative Study to Determine the Best Two-Dose Schedule and Route of Administration of Human Anthrax Vaccine: Final Study Report to the Food & Drug Administration. Fort Detrick, MD, U.S. Army Medical Research Institute of Infectious Diseases, 2000.)

experience with the skin test antigen in humans in western countries, and its utility in predicting immunity in humans remains unknown.

Correlates of Protection

After a naturally acquired infection, antibody to PA develops in 68% to 93% of cases as reported in different series, depending on the time when samples are drawn.[115,123,124,131] Antibody to LF occurs in 42% to 55% of cases, whereas antibody to EF is less frequently observed.[115,124] Antibody to the anthrax capsule occurs in 67% to 94% of cases.[124,131] This reaction contrasts with that of vaccinees, in which no response to capsule is expected because the vaccine strain is nonencapsulated. In the 2001 epidemic of inhalational anthrax, antibody to PA was detected in all confirmed survivors.

In experimental animals, there is generally a correlation between immunity and antibody titer to PA after immunization with the human vaccine.[132] However, the live veterinary vaccine provides significantly greater protection against anthrax in experimental animals than does the human vaccine, even though it often induces lower levels of antibody to PA,[105,108,109] suggesting that other antigens may be involved in protection. Thus the relationship between antibody to PA, as measured in these assays, and immunity remains obscure.

More recent studies using both live and protein-based vaccines have demonstrated a strong correlation between antibodies to PA and immunity. Barnard and Friedlander showed, for the first time using live vaccines producing varying amounts of PA, that protection was strongly correlated with antibody titers to PA,[133] a finding subsequently confirmed by Cohen and colleagues.[134] Pitt and colleagues, using the licensed human vaccine, found a similar in vitro correlation of immunity with antibody to PA, measured by ELISA and toxin neutralization, in a rabbit model of inhalational anthrax.[132] Reuveny and colleagues, using a PA vaccine to protect guinea pigs against an intradermal challenge, found that toxin neutralizing antibodies correlated better with survival than did antibodies measured by ELISA.[92] Further analysis of the antibody response to different epitopes on PA will further our knowledge of the nature of the protective antibodies.

Efficacy of Vaccine

The protective efficacy of different experimental PA-based vaccines that were derived from culture filtrates of *B. anthracis* was clearly demonstrated with the use of various animal models and routes of challenge.[101,113] A comprehensive, peer-reviewed evaluation by the National Academy of Sciences reported that "The committee finds that the available evidence from studies with humans and animals, coupled with reasonable assumptions of analogy, shows that AVA as licensed is an effective vaccine for the protection of humans against anthrax, including inhalational anthrax, caused by all known or plausible engineered strains of *B. anthracis*."[107]

A controlled clinical trial was conducted with a less potent vaccine similar to the currently licensed U.S. vaccine.[135] This field-tested vaccine was composed of an alum-precipitated, cell-free culture supernatant from an attenuated, unencapsulated, nonproteolytic strain of *B. anthracis*. This strain differed slightly from that used to produce the licensed vaccine and was grown under aerobic rather than microaerophilic conditions.[98] The study was conducted in a susceptible population working in four mills in the northeastern United States, where raw imported goat hair contaminated with *B. anthracis* was used. The results indicated that vaccination, compared with inoculation with a placebo, provided 92.5% protection against anthrax, combining the cutaneous and inhalational cases (95% confidence interval, 65% to 100%). No isolated assessment of

TABLE 31–3 ■ Efficacy of Anthrax Vaccines Against Inhalational Challenge in Rhesus Macaques

Reference	Vaccine Product	Vaccination
Wright et al. (1954)[98]	Alum-precipitated "preparation 138"	Two 1-mL SC doses 16 days apart
Darlow et al. (1956)[110]	Alum-precipitated vaccine	Two 1.25-mL SC doses 10 days apart
Ivins et al. (1996)[136]	Anthrax Vaccine Adsorbed	Two 0.5-mL IM doses 2 weeks apart
Pitt et al. (1996)[137]	Anthrax Vaccine Adsorbed	Two 0.5-mL IM doses 28 days apart
	rPA 50 μg + $Al(OH)_3$	Two 0.5-mL IM doses 28 days apart
Pitt et al. (1999)[138,*]	Anthrax Vaccine Adsorbed	Two 0.5-mL IM doses 28 days apart
Ivins et al. (1998)[106]	Anthrax Vaccine Adsorbed	One 0.5-mL IM dose
	rPA 50 μg + various adjuvants	One 0.5-mL IM dose
Fellows et al. (2001)[139]	Anthrax Vaccine Adsorbed	Two 0.5-mL IM doses 4 weeks apart

*Additional data from IM, intramuscular; LD_{50}, median lethal dose; rPA, recombinant protective antigen; SC, subcutaneous. (MLM Pitt, personal communication, 2002.)

the effectiveness of the vaccine against inhalational anthrax could be made because there were too few cases, although the only inhalational cases observed occurred in nonvaccinated individuals. This same vaccine previously was shown to protect rhesus monkeys against an aerosol exposure to anthrax spores.[21,107,118] A review of the methods and results of the trial noted above, as well as results of a trial with the live spore vaccine developed in the former Soviet Union, concluded that both products were effective.[118]

There have been no controlled clinical trials in humans of the efficacy of the currently licensed U.S. vaccine, although the differences between the BioPort vaccine and the PA-based vaccine used in the Brachman et al. study[135] are minor from a regulatory perspective.[107] The BioPort vaccine has been tested extensively in animals and has protected guinea pigs against both an intramuscular[108,109] and an aerosol[105] challenge. More recent experiments show that this vaccine also protected rhesus monkeys against a lethal aerosol challenge with anthrax spores.[106,136–139] Inhalational challenge studies in nonhuman primates vaccinated with either the licensed human vaccine or a recombinant PA vaccine are summarized in Table 31–3.

Duration of Immunity

The duration of immunity induced by vaccination has not been clearly established. In the field trials that evaluated a vaccine similar to the currently licensed U.S. vaccine, one case of cutaneous anthrax occurred 5 months after the initial three-dose series and just before the scheduled 6-month boosting dose.[135] Although data are insufficient to support any firm conclusions, this observation suggests that the immunity induced by the initial series of three doses of the current vaccine may not be long lasting and that the recommended schedule of annual boosters is necessary. Ongoing studies of clinical correlates of protection that involve reducing the total number of vaccine doses by using longer intervals between booster doses, and using an intramuscular rather than subcutaneous route of injection, will help clarify this point.[135a]

Postexposure Prophylaxis with Vaccination

Postexposure vaccination by itself is unlikely to be of any benefit because of the short incubation period and the rapid course of the disease. Animal studies support this conclusion.[21] However, vaccination combined with antibiotic prophylaxis before the onset of clinical illness may offer the best possible protection against inhalational anthrax after an aerosol exposure. This is because of the unusual propensity of anthrax spores to persist in the host for long periods and possibly germinate after antibiotics have been discontinued.[18–21] Vaccination will allow for the development of an immune response during the period of antibiotic prophylaxis. Thus postexposure vaccination may shorten the period of antibiotic prophylaxis required for protection.

This recommendation is supported by a National Academy of Sciences report that concluded that

> *these limited data suggest that the use of vaccine in combination with an appropriate antibiotic for 30 days could provide excellent postexposure protection against inhalational anthrax. Although the additional benefit from receiving the vaccine after a prolonged period of antibiotic use is not*

Challenge Dose	Challenge Strain	Vaccination to Challenge	Time from Last Survival	
			Vaccinated Animals	Unvaccinated Animals
39,000–82,000 spores	Vollum	16 days	3/4	0/4
890,000 to 3 million spores	Vollum	34 days	4/4	0/2
10–15 LD_{50}	M.36 Vollum	7 days	10/10	0/10
10–15 LD_{50}	M.36 Vollum	1 yr	10/10	0/10
10–15 LD_{50}	M.36 Vollum	2 yr	6/7	1/9
255–760 LD_{50}	Ames	8 wk	10/10	0/5
161–247 LD_{50}	Ames	38 wk	3/3	
239–535 LD_{50}	Ames	100 wk	7/8	0/2
899 ± 62 LD_{50}	Ames	3 mo	9/9	0/2
899 ± 62 LD_{50}	Ames	3 mo	9/10	
133 ± 43 LD_{50}	Ames	3 mo	5/5	0/6
74.4 LD_{50}	Ames	6 wk	10/10	0/3
78–117 LD_{50}	Ames	6 wk	28/29	
398 LD_{50} (Ames equivalent)	ASIL K7978-Namibia	6 wk	10/10	0/2
1004 LD_{50} (Ames equivalent)	ASIL K9729-Turkey	6 wk	8/10	0/2

proven, reliance on the vaccine alone after exposure is clearly insufficient, as some protection is needed during the time required for an immune response to develop.[107]

Vaccine Safety

Common Adverse Effects

Studies of an earlier PA-based vaccine used in the human field trial showed that, during the initial series of three subcutaneous injections, the incidences of systemic and of significant local reactions were 0.7% and 2.4%, respectively.[98,135] An increase to 1.3% and 2.7%, respectively, was noted with the booster doses. A more detailed study showed that local reactions increased in frequency up to the fifth inoculation and then declined.[135] In this study, there was a 0.2% incidence of systemic reactions and a 2.8% overall incidence of significant local reactions. Systemic reactions include mild generalized myalgia, slight headache, and mild to moderate malaise for 1 to 2 days. Most local reactions are mild, consisting of 1 to 2 cm of erythema and slight local tenderness appearing the first day and disappearing within 1 to 2 days. Significant local reactions consist of induration, erythema greater than 5 cm in diameter, edema, pruritus, local warmth, and tenderness. These reactions are maximal at 1 to 2 days after vaccination and usually disappear over 2 to 3 days. Very rarely, the edema may be extensive and extend from the deltoid to the forearm. A small, painless nodule at the injection site, persisting for several weeks, also has been observed, but only rarely. Severe local reactions were observed in individuals with a history of cutaneous anthrax who were inadvertently immunized.[98] All local reactions resolved without complication.

The licensed aluminum hydroxide–adsorbed PA vaccine, when first used, gave an incidence of local reactions similar to that of the alum-precipitated vaccine, although no detailed observations were reported.[99] In an open-label study from 1966 to 1971 with the licensed vaccine, approximately 7000 textile employees, laboratory workers, and other at-risk individuals received 15,907 doses.[140,141] There were 24 reports (0.15% of doses) of severe local reactions (defined as edema or induration >120 mm in diameter or marked limitation of arm motion or axillary node tenderness). There were 150 reports (0.9%) of moderate local reactions (edema or induration 30 to 120 mm in diameter) and 1373 reports (8.6%) of mild local reactions (erythema only or induration <30 mm in diameter). Four cases of systemic reactions (<0.06%), reported as transient, included fever, chills, nausea, and general body aches.

The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) assessed the safety of the licensed anthrax vaccine as part of a randomized clinical study between 1996 and 1999.[125] A total of 28 volunteers received subcutaneous doses according to the licensed schedule. Each volunteer was observed for 30 minutes after administration of AVA and scheduled for follow-up evaluations at 1 to 3 days, 1 week, and 1 month after vaccination. Four volunteers reported seven acute adverse events within 30 minutes after subcutaneous administration. These included erythema (3), headache (2), fever (1), and elevated temperature (1). Of these events, a single patient reported simultaneous

occurrence of headache, fever, and elevated temperature (100.7°F). The most common local reactions reported were tenderness, erythema, subcutaneous nodule, induration, warmth, local pruritus, limited arm motion, and edema. Local reactions were found to occur more often in women. No abscess or necrosis was observed at the injection site. Systemic reactions included malaise, headache, myalgia, fever, anorexia, respiratory difficulty, and nausea or vomiting (4%). All local and systemic adverse events reported in this study were transient in nature. There was one report of a delayed-type hypersensitivity reaction beginning with lesions 3 days after the first dose.

USAMRIID also analyzed the occupational health records of 1583 workers (1249 men and 334 women) who reported adverse events after receiving 10,722 doses of the licensed anthrax vaccine from 32 separate vaccine lots.[142] Of this group, 273 people received 10 or more doses and 46 people received 20 or more doses. With regard to injection-site reactions, 3.6% of doses were reported to produce redness, induration, itching, or edema. Most people who reported a reaction received subsequent doses without problems, but the subset of people who reported an injection-site reaction were more likely to report a local reaction to a later dose. Systemic events of headache, fever, chills, malaise, and muscle or joint aches were reported after 1% of doses. The most common of these were headache (0.4%), malaise (0.4%), and fever (0.1%). Women noted both local (i.e., erythema, induration, edema, swollen lymph nodes, lumps) and systemic (i.e., headache, fever, dizziness, hives) events more commonly than men. Vaccine recipients less than 40 years old reported adverse events more often than those 40 years or older.

Two uncontrolled case series used self-administered surveys to assess anthrax vaccine safety. Among 601 health care workers at an Army hospital in Honolulu,[107,143] women reported more localized itching (56% to 68%) than did men (24% to 31%). Women developed more subcutaneous nodules (81% to 93%) than did men (56% to 65%). Moderate to large injection-site reactions (erythema >10 cm) were more common among women (40% to 51%) than men (17% to 32%). Women reported more swelling of the lower arm (8% to 15%) than did men (7% to 10%). About 20% of men and women reported symptoms that they personally judged could be ignored; 15% reported symptoms that affected their activity for a short time, but did not limit their ability to perform duties; 8% reported symptoms that affected their activity for a short time that were relieved by self-treatment with nonprescription medication; and less than 2% reported symptoms unrelieved by medication, with their ability to perform duties limited for a short time. From 1.5% to 2.7% of women and from 1.2% to 2.1% of men reported systemic events leading to limitation of performing duties. Events in both genders were similar in resolving on their own over the course of a few days without residual consequences.

The other uncontrolled case series was at a U.S. Army base in South Korea.[107,143] Participants included 2214 men and 610 women given the licensed anthrax vaccine. Women reported subcutaneous nodules more frequently (50% to 62%) than men (21% to 29%). Erythema greater than 12 cm in diameter was self-reported by 2% to 4% of women and 0.4% to 1% of men. Women experienced more itching at the local site (20% to 37% vs. 6% to 8%); fever (2% to 4% vs. 1%), chills (3% to 6% vs. 1% to 2%), and malaise (8% to 15% vs. 4% to 7%) than did men. Overall, 0% to 1.9% reported that their work activity had been limited to some extent or were placed on limited duty. From 0% to 1.1% reported losing 1 or more days of duty; 0.4% to 1.7% consulted a clinic for the reaction. Regardless of gender, almost all reported events were localized or minor and self-limited and did not lead to impairment of work performance.

Rare Adverse Effects

The best evidence evaluating the overall safety of the licensed vaccine comes from database studies from the Army Medical Surveillance Activity and the Naval Health Research Center.[107,144] These studies established that anthrax-vaccinated and unvaccinated personnel of either gender are hospitalized and visit outpatient clinics at the same rates overall for each of 14 major categories within the International Classification of Diseases, and for each of several specific diagnoses with speculative association with anthrax vaccination. For example, 1 per 35 anthrax-vaccinated people is hospitalized each year, compared to 1 per 28 unvaccinated people being hospitalized per year.

Three reports described the long-term health of 99 male laboratory workers who received a variety of licensed and investigational vaccines (volume range, 52 to 134 mL), including both the early and the current formulation of anthrax vaccine.[145–147] The third study included a small control group. Although there were elevations in liver and kidney function tests and white blood cell counts in some of these men, none developed any unusual diseases or unexplained symptoms that the authors attributed to repeated doses of multiple vaccines.

All reports to the Vaccine Adverse Event Reporting System (VAERS) involving the licensed anthrax vaccine were evaluated by the Anthrax Vaccine Expert Committee (AVEC), composed of civilian physicians.[148] The AVEC evaluated 1857 VAERS reports and additional medical records corresponding to 1793 recipients of the licensed anthrax vaccine between March 1998 and February 2002. The 1857 adverse event reports can be grouped into three main categories, based on effect on the vaccine recipient's functional status: hospitalization, inability to work for 24 hours or more, and "other." Sixty-four of the 1857 reports involved hospitalization. The civilian panel found that 11 of the 64 "very likely/certainly" or "probably" were caused by anthrax vaccine. All 11 involved allergic or inflammatory reactions at the injection site. Another 172 reports involved inability to work for 24 hours or more (but did not involve hospitalization); 94 of the 172 certainly or probably were caused by anthrax vaccine. These 94 reports primarily described injection-site reactions, various rashes, acute allergic reactions, and viral-like symptoms. The balance of the 1857 reports, 1621, involved neither hospitalization nor time off work for 24 hours or more. All were reviewed by the AVEC, which found no patterns of unexpected adverse events.

Two cases of optic neuritis were reported in soldiers subsequent to anthrax vaccination. Optic nerve antibodies were found in one case, but no epitopes were found in common between optic nerve and the anthrax organism.[148a]

A cohort study involving 4092 active-duty women in the U.S. Army assessed the effect of the licensed anthrax vaccine on pregnancy and childbirth.[149] This cohort contrasted 3135 women vaccinated against anthrax and 957 unvaccinated women, with 39,549 person-months of

follow-up. The anthrax-vaccinated and unvaccinated women had an equivalent likelihood of becoming pregnant, as well as giving birth. The study found no differences in birth outcomes between the two groups, but the study did not have adequate statistical power to rule out a small effect of vaccination on adverse birth outcome, given the low number of adverse outcomes.

These and other safety studies of anthrax vaccine, some still in the peer-review process before publication, were critically reviewed by the expert committee convened by the National Academy of Sciences.[107] This comprehensive, peer-reviewed report concluded that the licensed anthrax vaccine has a side effect profile similar to that of other adult vaccines. According to the reviewers,

> *The committee found no evidence that people face an increased risk of experiencing life-threatening or permanently disabling adverse events immediately after receiving AVA, when compared with the general population. Nor did it find any convincing evidence that people face elevated risk of developing adverse health effects over the longer term, although data are limited in this regard (as they are for all vaccines)."*[107]

A list of studies showing the safety of AVA are given in Table 31–4.

Indications for Vaccination

Routine immunizations are recommended for industrial workers who handle potentially contaminated animal products, including wool, goat hair, hides, and bones imported from countries in which animal anthrax continues to occur. These countries are primarily in Asia and Africa but are occasionally in South America or the Caribbean. A veterinarian or agricultural worker who has contact with potentially infected animals should be immunized, as should laboratory workers who work with *B. anthracis*.[70]

Special circumstances that warrant vaccination with anthrax vaccine include a threat of biologic warfare or terrorism. The U.S. Armed Forces began vaccinating some service members in 1998 to protect against anthrax arising from the use of *B. anthracis* as a biologic weapon.

Contraindications and Precautions

A contraindication to being vaccinated is a hypersensitivity reaction to the vaccine. This is uncommon, but several individuals who have received the initial dose or doses developed moderately severe local reactions with some systemic response. If it is necessary to immunize such individuals, pretreatment with antihistamines and nonsteroidal anti-inflammatory drugs may be of value, although this has not been evaluated scientifically.[70]

Public Health Considerations

The use of the anthrax vaccine in industrialized populations had a significant impact on the reduction of natural anthrax cases among industrial workers and is one of the main methods by which industrial anthrax was controlled. Improvements in the industrial environment, with better manufacturing equipment and environmental control, also have helped reduce the industrial risks of naturally occurring anthrax. Additionally, replacement of animal products (primarily goat hair) with synthetic fibers has had a favorable impact on the occurrence of anthrax infection. It is ironic that mail-processing machines replaced wool-processing machines as a source of industrial risk in the 2001 anthrax bioterrorism attacks.

Agricultural cases have been reduced by control of the disease in animals through the use of animal vaccines. The routine immunization of animals in areas with continuing cases of animal anthrax and the immunization of appropriate humans agriculturally and industrially exposed to *B. anthracis* will serve to reduce the number of naturally occurring human cases.

Future Vaccines

Current acellular vaccines against anthrax are less than ideal for several reasons. These vaccines are composed of an incompletely defined culture supernatant adsorbed to aluminum hydroxide. There is only partial quantification of

TABLE 31–4 ■ Anthrax Vaccine Safety Studies

Study	Number of subjects
Brachman (1962)[135]	379
CDC observational study, review by FDA, advisors[140]	6986
Fort Detrick multidose, multivaccine safety studies[145–147]	99
Fort Detrick special immunization program (2001)[142]	1583
Fort Bragg booster study (after Persian Gulf War)[128]	281
USAMRIID reduced-dose/route-change study (2001)[125]	173
Canadian Forces safety survey[107]	576
TAMC-600 survey (Tripler Army Medical Center)[107,143]	603
U.S. Forces Korea records (2000)[107,143]	2824
USAF visual acuity study[107]	958
USAF Air Combat Command study, Langley Air Force Base[163]	5187
VAERS review by Anthrax Vaccine Expert Committee (2002)[148]	1793
Inpatient/Outpatient cohort study[107,144,164]	350,296 person-yr

CDC, Centers for Disease Control and Prevention; FDA, Food and Drug Administration; USAF, U.S. Air Force; USAMRIID, U.S. Army Medical Research Institute of Infectious Diseases; VAERS, Vaccine Adverse Event Reporting System.

the PA content of the vaccine or other constituents, so the degree of purity is not fully known. Standardization is determined by biologic activity in an animal potency test. Studies in progress will determine the extent to which administering this aluminum-adsorbed vaccine subcutaneously (rather than intramuscularly) is responsible for the observed rate of injection-site reactions. The currently licensed schedule is less than optimal, in that six doses are required over 18 months, followed by annual boosters. A simpler vaccination schedule with fewer doses is also being evaluated. Although there is evidence that the efficacy of the vaccine against parenteral challenge of rodents may be less against some strains of anthrax than others,[108,109,115] the vaccine protected rhesus monkeys against a more rigorous aerosol challenge with all strains tested, including those overcoming resistance in rodents.[136,139] Clearly, the ideal anthrax vaccine would be more completely defined and less reactogenic, and able to produce long-lasting immunity within 30 days.[107]

Further understanding of the molecular pathogenesis of anthrax and of the structure of the PA and its interaction with LF and EF can be expected to lead to significant progress toward the development of improved vaccines. For example, genetically defined mutations in the receptor-binding domain,[150,151] the protease-sensitive domain,[152] or other parts of the molecule[153] may generate a less toxic PA preparation to be used either alone or as a complex with edema or lethal factor. Similarly, mutations in either the edema or lethal toxin may allow evaluation of nontoxic complexes with PA. Evidence in experimental animals suggests that adjuvants other than aluminum may increase the protective efficacy of PA substantially even after a single dose,[154,155] and that new formulations using microcapsules also may be of value.[156]

Several vaccine candidates based on recombinant PA protected rhesus monkeys from inhalational challenge.[104,106,157,158] These vaccines are in the most advanced stages of development and are undergoing final preclinical testing before beginning Phase I human trials.

Another approach has been to develop live vaccines for human use, because several reports demonstrated that a live vaccine protects experimental animals better than does the licensed human PA vaccine.[105,108,109,157] The precedent exists for using such a vaccine in humans in Russia and the former Soviet republics. Live vaccines that are known to protect experimental animals against anthrax include aromatic compound–dependent, toxigenic, nonencapsulated strains of *B. anthracis*,[155] as well as *B. anthracis*,[133] *B. subtilis*,[157] *Salmonella*,[159] and vaccinia,[160] each constructed to contain the cloned PA gene. Finally, other approaches using PA or LF have included nonreplicating DNA vaccines[161] (C Schmaljohn, personal communication, 2002) and viral replicons (JL Lee, personal communication, 2002).

Attempts to identify antigens other than the PA of the toxin that may contribute to protection are also underway. Spore components[134,162] and the capsule (AM Friedlander, personal communication, 2002) have been shown to offer additional protection in some small-animal models. In addition, the forthcoming completion of the *B. anthracis* genome is anticipated to advance the search for new vaccine candidates as well as therapeutic targets.

Although these efforts are in the experimental stage, they may lead to the production of a vaccine that is less reactogenic, requires fewer doses, and provides more effective and long-lasting immunity.

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