

Anthrax

by
Elizabeth Gray

College of Medicine Class of 2007
Medical University of South Carolina
Molecular Basis of Medicine Treatise

Abstract

Anthrax is an ancient bacterial infection capable of infecting nearly all warm-blooded animals. It has existed since early-recorded history as a disease of both human beings and their livestock. However, recent exposures to anthrax due to bioterrorism have turned anthrax and its mechanisms of infection into an important topic of inquiry. As new information is obtained, more effective treatments for anthrax are being made possible.

Gray, Elizabeth. Anthrax. South Carolina Journal of Molecular Medicine (SCJMM) 5:6-10; 2004.

Author's Addresses:

grayel@musc.edu

Key Words: anthrax, lipid rafts, bioterrorism

The manuscript has been seen and approved by the author and the editor.

Introduction

As long as man has been dependent on livestock and animal husbandry for survival, anthrax has existed as a health threat. Accounts ranging from as far back in history as the Old Testament and the Roman poet Virgil describe a disease that the average modern American, until recent events, had all but forgotten. Unfortunately, the deliberate distribution of anthrax as a biological weapon and act of terrorism reintroduced anthrax into everyday vocabulary as a topic of concern. The scientific community also responded by continuing efforts to discover new, more effective ways to prevent and treat anthrax infection.

The bacterium *Bacillus anthracis* produces toxins that create the symptoms known as anthrax. Several forms of the infection exist with distinct progressions and mortality rates. These include cutaneous, inhalational,

and gastrointestinal anthrax which are distinguished by whether spores enter the body through the skin, the lungs, or the intestinal tract respectively. Cutaneous anthrax, the most common and least fatal form of the infection, is transmitted through openings in the skin or by biting flies. It begins as a papule that grows to a 1 cm vesicle that then ruptures and develops into the black eschar from which it gets its name from the Greek word for "black coal" (Sternbach 2003). This form can become systemic if not treated, in which case it is much more likely to be fatal. Inhalational anthrax is the second most common form of infection and also much more severe. Usually contracted through textile and tanning industry workers, this "wool sorter's disease" presents itself in two stages (Sternbach 2003). The first stage follows the progression of the flu with malaise, fatigue, nonproductive cough, and fever.

The second stage, which might be preceded by a brief alleviation of symptoms, involves acute respiratory distress, cyanosis, shock, diaphoresis, and often ends in death. Without treatment this form of the disease is 95% fatal (Cranmer 2003). The third form of infection, gastrointestinal anthrax, is the least common. Contracted from eating contaminated and under cooked meat, symptoms include abdominal pain, fever, nausea, diarrhea, and vomiting. Edema of the GI tissue can lead to shock. This form is fatal 50% of the time and requires treatment and forced fluids.

Although endemic to certain areas in Asia and Africa, anthrax in America was all but nonexistent until its deliberate distribution in 2001 (Cranmer 2003). The toxin responsible for the deadly effects of *Bacillus anthracis* is comprised of three distinct proteins that interact with one another in order to enter and infect cells. The bacteria itself is protected from phagocytosis by a polyglutamyl capsule and a dormant, persistent spore resistant to many extremes of heat, moisture, UV radiation and even to some types of disinfectants. Drugs aimed at destroying the bacteria often fail to inhibit the activity of the toxins responsible for the actual symptoms of the disease. Current research focuses on the mechanisms by which this deadly toxin crosses cell membranes and invades host cells in hopes of developing a more powerful, more reliable, and more effective treatment for anthrax.

Discussion

Anthrax describes the disease caused by infection of the spore forming, gram-positive bacterium, *Bacillus anthracis*. The bacterial spores prove to be resistant to heat, ultraviolet light, gamma radiation, drying, numerous disinfectants and can stay dormant for years. The bacterium has a diameter of 1-1.5 μm , a length of 3-10 μm ,

and grows as gray-white colonies measuring 4-5 mm in culture (Cranmer 2003). It contains two plasmids, pXO1 and pKO2, responsible for its toxicity. pXO1 codes for the genes that make up the trio of toxins that invade cells, causing the symptoms and possibly even death associated with anthrax infection (Dixon 1999). pXO2, however, codes for three genes responsible for producing the polyglutamyl capsule that protects the bacterium from phagocytosis by macrophages of the host's immune system (Dixon 1999). The toxins produced by pXO1 also inhibit the immune response, increasing the bacteria's resistance to the host's defenses and, by extension, its lethality.

The toxins produced by pXO1 cause the symptoms associated with anthrax infection. Researchers have focused on the entry of these toxins into cells and their mechanisms of infection as areas for treatment, vaccination, and prevention. Three distinct proteins make up the toxin complex necessary for invading and infecting cells. These proteins are edema factor (EF), lethal factor (LF), and protective antigen (PA). EF is a calmodulin dependent adenylate cyclase that increases intracellular levels of cAMP (Abrami 2003). These increased levels compromise the cell membrane's control of ion and water transport, causing edema. This factor also inhibits phagocytes it invades that would otherwise attack the infecting bacteria before it could cause severe illness and death (Young 2002). The fluid imbalance can lead to shock and other symptoms precluding death as well. LF is a zinc metalloprotease that targets mitogen-activated protein kinase kinase and causes macrophage cell death. The macrophages release tumor necrosis factor α and interleukin 1- β , responsible for the severity of systemic anthrax (Dixon 1999). The final toxin, PA, is necessary for the other

two to access the cell and begin their deadly metabolism.

Anthrax exists as such a threatening disease because by the time symptoms appear and a person suspects infection, the bacteria has already multiplied to as many as 10^7 or 10^8 organisms per milliliter of blood (Dixon 1999). At this point, administrative of antibiotics such as penicillin, ciproflaxin, or doxycycline will combat the bacteria, but the toxins produced by these bacteria before this treatment will continue to attack cells and possibly remain fatal. Anthrax vaccine adsorbed, AVA, a vaccine given predominantly to soldiers involves six shots over 18 months, booster shots, and there are limited amounts (Young 2002). There is a need for more reasonable and available vaccines and treatments to combat the bacteria and its toxins in large-scale populations if anthrax continues to be a possible terrorist threat. Fortunately, researchers have discovered many areas to explore for the development of drugs that can combat the toxins while antibiotics rid the body of the bacteria.

The EF and LF toxins begin their journey into the cell when PA, an 83-kD protein, binds to anthrax toxin receptor (ATR), a common, 368 amino acid, type one membrane protein (Bradley 2001). Once bound to the cell membrane, a protease of the furin family cleaves the protein at the cell's surface, releasing an NH_2 -terminal 20-kD fragment. The 63-kD carboxyl end remains attached to the membrane and is referred to as PA63. Only once PA has been fragmented to its PA63 form can it oligomerize to form ring shaped heptamers. LF and/or EF can only interact with the cell surface once this heptamerization has taken place. The PA63 and LF and/or EF complex is then taken into the cell and transported as an endosome through clathrin dependent endocytosis (Abrami 2003). Low pH initiates the formation of channel by the

complex through which the LF and EF factor presumably enter the cell and begin their respective pathways. This mechanism provides numerous opportunities for drug interactions that could halt the debilitating effects anthrax by preventing the toxins from ever even entering the cell.

One area researched by Abrami and colleagues is the formation of the PA63 heptamers without which the other toxin factors could not bind and enter the cell. Abrami's research group provided convincing evidence that it is the formation of this PA63 heptamer that connects PA63 with lipid rafts of the cell membrane. Lipid rafts are cholesterol and glycosphingolipid rich areas of the cell membrane capable of lateral movement that act in signal transduction, endocytosis, infection processes, and cholesterol homeostasis (Abrami 2003). Lipid rafts can be detected as areas of the cell membrane resistant to nonionic detergents at 4°C and can be purified on density gradients. Using this property of lipid rafts, Abrami demonstrated that PA63 is associated with them by confirming its presence in low density fractions with lipid rafts and its absence when cholesterol or sphingomyelin were removed and lipid rafts could not form. Furthermore, it is the clustering of ATR that creates lipid rafts as demonstrated by an experiment where antibody cross-linking of ATR formed lipid raft domains. This clustering is accomplished by anthrax toxin through the heptamerization of PA63. Experiments also demonstrated the association with EF and LF to these lipid raft domains, a logical conclusion since these toxins only bind to the clustered PA63 heptamers. Abrami demonstrated that the insertion of these toxins into the cell depends on the formation of lipid rafts and provides a likely site for drug interactions. Several avenues of drug development have already begun. A group at Harvard has

worked on constructing a polypeptide chain that will “block” the heptamer before the toxins can bind to it (Young 2002). Another method under exploration is the prevention of an active form of the heptamer by inserting mutant forms of PA, called dominant negative inhibitors (DNI), that disrupt the heptamer’s function (Young 2002). Ahuja and colleagues incorporated protective antigen with Asp425 and Phe427 deletions into mice with wild type PA in a ratio of 1:8 and found that the mutants protected the mice from infection. They believe that just one mutant PA molecule is necessary to inactivate the entire heptamer that incorporates it, preventing EF and LF toxicity (Ahuja 2003). Inhibitors such as this provide a likely basis for future treatment. They will elicit an immune response to the bacteria while also preventing the toxic effects of the toxins (Young 2002). Finally, scientists are also exploring the use of soluble anthrax toxin receptors (sATR) that would bind to protective antigen extracellularly before it could bind to a host cell (Young 2002).

Conclusion

Research into the mechanisms by which toxins of *Bacillus anthracis* invade host cells has provided insight into various opportunities for drug treatment. Detailed knowledge of the structures of edema factor, lethal factor, protective antigen, and how these three factors interact to cause infection have served as a basis for more effective drugs specified to treat anthrax. Researchers have already begun to explore compounds capable of preventing protective antigen from binding to anthrax toxin receptors, of blocking the formation of lipid rafts, and of inhibiting the entry of anthrax toxins into cells. Eventually, some of these compounds might become the future of anthrax therapy. As the mechanisms of lipid-raft formation and infection continue to be elucidated,

more effective drug therapies for anthrax and a greater understanding of bacterial infection will be the reward.

References

1. Abrami, L. *et al.* “Anthrax toxin triggers endocytosis of its receptor via a lipid raft-mediated clathrin-dependent process.” *J. Cell Biol.* February 3, 2003 **160**:3 pp 321-8.
2. Ahuja, Nidhi *et al.* “Deletion mutants of protective antigen that inhibit anthrax toxic both in vitro and in vivo.” *Biochemical and Biophysical Research Communications.* August 1, 2003 **307**: 3 pp 446-450.
3. Bradley, K.A. *et al.* “Identification of the cellular receptor for anthrax toxin.” *Nature.* 2001 **414** pp 225-229.
4. Bull, James J. and Colin R. Parrish. “A Binding Contract for Anthrax.” *Science.* July 12, 2002 **297** pp 201-202.
5. Cranmer, Hilarie. “CBRNE- Anthrax Infection.” *Emedicine.* January 21, 2003
6. Dixon, Terry C. *et al.* “Anthrax.” *The New England Journal of Medicine.* September 9, 1999 **341**:11 pp 815-826.
7. Gupta, Pradeep K. *et al.* “Conformational fluctuations in anthrax protective antigen: a possible role of calcium in the folding pathway of the protein.” *FEBS Letters.* November 20, 2003 **554**:3 pp 505-510.
8. Kliewer, Steven A. “Anthrax mounts a nuclear attack on glucocorticoid signaling.” *Trends in Pharmacological Sciences.* November 2003 **24**:11 pp 558-559.
9. Kurzchalia, Teymuras. “Anthrax toxin rafts into cells.” *J. Cell Biol.* February 3, 2003 **160**:3 pp295-296.
10. Nassi, Shilla *et al.* “PA63 Channel of Anthrax Toxin: An Extended β -Barrel.” *Biochemistry.* 2002 **42**:5 pp 1145-1150.

11. Petosa, Carlo *et al.* “Crystal Structure of the anthrax toxin protective antigen.” *Nature*. February 27, 1997 **395** pp 833-835.
12. Sternbach, George. “The history of anthrax.” *J. of Emergency Medicine*. May 2003 **24**:4 pp 463-467.
13. Young, JA and J.R. Collier. “Attacking Anthrax.” *Scientific America*. March 2002, **286**:3 pp 48-50, 54-59.

Editor: Maurizio Del Poeta, MD
Selective 2003: Lipid-mediated Infectious Diseases