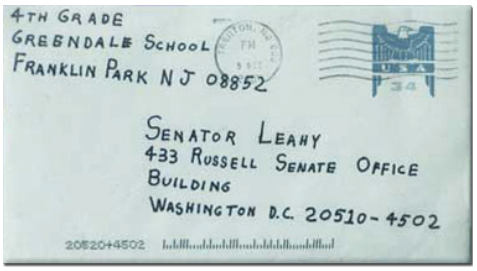
Biological Terrorism: The Anthrax Scare of 2001



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Part I—The Anthrax Scare of 2001

February 2003, the US Postal Service Brentwood Processing and Distribution Center, Washington, DC

Janice smiled at the nine postal workers seated in front of her. "Hi, I'm Janice Blanchard, and this is my colleague Yolanda Haywood. This is Amy Lundgren, and she's our ASL interpreter for today. Thanks for agreeing to participate in our focus group."

"Hey, for fifty bucks and free food?" said a middle-aged man, tucking into a pastry. "No problem!"

Janice smiled. "We'd like to ask you some questions about whether you thought that communication following the discovery of the anthrax letters in your facility was effective. We're taping the session so Yolanda and I can analyze the results of this group and others, but your comments will be held in strict confidentiality, so we hope you'll feel that you can speak freely."

"Are you kidding?" retorted a woman whose name tag identified her as Tanya. "Management didn't give us the information we needed. I didn't know anything at all about anthrax. I got most of my information from the evening news! And I never did hear about the results of my nasal swab."

A young woman signed rapidly, and Amy interpreted her sentiments to the group. "I didn't know what was going on and why everyone was leaving the building. I got an e-mail from a friend and she told me I should turn on the news. Then someone wrote me a note and told me that we were being evacuated, and I didn't know why. I really felt left out with everything that was going on. And later on, I know that the hearing people got more information than I did. They could ask questions and I couldn't."

A man named Michael snorted. "Yeah, and did you notice that they evacuated the Senate long before they closed us down? It's because they're all white folks up there."

Tanya nodded in agreement. "And I had a positive view of the CDC before this happened, but not any more. I thought they were supposed to know all about diseases, but I kept getting different answers from different people. If they didn't know something, they should just say so. I had the impression that the CDC thought that we were all guinea pigs and this was just an interesting experiment, rather than being concerned about us as people and for our health."

Janice looked up from her notes. "Did you get any information from your personal physicians? What about the course of antibiotic treatment? Did any of you get vaccinated?"

"My doctor didn't really know that much about anthrax," said Tanya. "And no, I didn't even finish taking the Cipro they gave us. There were too many side effects, and I was working in a different part of the facility and I figured I probably hadn't been exposed."

 Image credit: One of several letters sent from Trenton, New Jersey, laden with anthrax spores. Federal Bureau of Investigation (FBI), http://www.fbi.gov/pressrel/pressrel01/102301.htm.

Michael added, "No way was I going to get vaccinated. My brother is in the military and he said the side effects were just awful. And on TV, it sounded like the vaccine was experimental. I don't want to be a guinea pig, no thank you!"

Tanya sighed. "They should just appoint one person to be a liaison, and give us honest information. This was really stressful. I hope it doesn't happen again."

August 2003, Center for Biodefense and Emerging Infectious Diseases, UTMB, Galveston, TX

"Dr. Matthews, Edwin Hammer on line 1." A.J. Matthews heard the voice of his secretary and groaned. He had been ducking Hammer's calls and suspected he couldn't keep doing that for much longer. "Put him through," he answered, and braced himself for Hammer's accusations.

"Edwin Hammer, Sunshine Project, how are you today Dr. Matthews? We're still waiting for the minutes of the Institutional Biosafety Committee meeting. You know that since you receive NIH funding those records are public."

"Look Ed, I'm all for transparency, but I have to obey the law here—the Patriot Act and the Homeland Security Act have rules about what kind of information can be published and what can't."

"You know as well as I do that the only information you're not allowed to publish is the quantities and locations of biological agents, and combinations of keypads and locks, and such. You're deliberately trying to be secretive about what you're doing, and you're awash with funding for all of your projects. This is public money and the public has the right to know what you're doing."

"Ed, if we release details about our biodefense research, the terrorists could get a hold of it! Then where would we be?"

"Sorry, but that argument just doesn't hold water. Being secretive might lead other countries to believe that we're developing biological weapons, and that will just encourage them to do the same. Besides, the entire nuclear industry has been top-secret, and that hasn't stopped other countries from developing nuclear weapons, has it?"

Before A.J. Matthews could think of a response, Hammer abruptly ended the conversation. "You'll be hearing from me again," he said firmly before hanging up the phone.

March 2005, The Ministry of Health Office, Cambodia

Ministry of Health official Ly Sovann mopped his brow. Goodness, it was hot, and it was only March. His mobile rang, for the umpteenth time that morning. "Yes?"

He heard the panicky voice of one of his health workers on the other end of the line. A woman in a remote village had died two months ago of avian influenza, and since then health workers suspected "bird flu" whenever one of their clients developed flu-like symptoms.

Ly Sovann tried to speak calmly. "Yes, I suppose it could be bird flu, but it could also be diarrhea or measles," he said, trying to be reassuring. But the threat of bird flu was very real, and his yearly budget for educating the 13 million people in his country was just $2,500. He remembered how his grandparents had spoken about the 1918 flu pandemic that killed 20-40 million people in Southeast Asia. "I really hope something like that doesn't happen again," he thought to himself as his phone rang again.

References

These stories are a fictionalized account of actual events and are based on published papers and news reports. Actual focus groups were conducted by Janice Blanchard and Yolanda Haywood of the George Washington University Department of Emergency Medicine. Blanchard, Haywood, and their colleagues published their findings in the *American Journal of Public Health*, 95(3), March 2005, pages 489–495. The Sunshine Project was described in the *Galveston Daily News*; the article can be found at
http://galvestondailynews.com/story.lasso?ewcd=93d56792662f9c22
Minister of Health Ly Sovann's story can be found at
http://www.zkea.com/news_archive.html?ARCHIVE=05-03-03

Part II—Project Design

A number of "interest groups" have been defined for this case study. They are: biochemists, immunologists and microbiologists. Each student in the class will be assigned to an interest group. Students in each group will assume the role of the interest group assigned them. They will read the assigned papers (listed below) and prepare a group oral report to present to the class. Presentations should be about 30 minutes long, with the remaining 20 minutes devoted to class discussion. At the conclusion of the discussions, each student will write a 1–2 page position paper.

Schedule

Day 1: Introduction to anthrax (presentation by instructor). Answers to common questions must be turned in at the beginning of class. We will discuss the answers to the common questions and we will also frame our debate by listening to a portion of "The Connection" radio show broadcast on March 17, 2005: "Is Biodefense the Best Defense?"

Day 2: Biochemists' presentation and discussion

Day 3: Immunologists' presentation and discussion

Day 4: Microbiologists' presentation and discussion

Day 5: Position paper due. In a 1–2 page paper, please offer concise answers to the following:

(a) What is the threat of anthrax to American civilians?

(b) Should all Americans be vaccinated against anthrax?

(c) Should Cipro be given prophylactically during situations such as the anthrax mailings of October 2001?

(d) Is the funding for biodefense research appropriate, and if so, should results be kept secret?

Common Papers

All students should read the following papers:

Mourez, M., Lacy, D.B., Cunningham, K., Legmann, R., Sellman, B.R., Mogridge, J., and Collier, R.J. (2002) "2001: A year of major advances in anthrax toxin research" *Trends in Microbiology,* 10(6):287–293.

Collier, R.J., and Young, J.A.. (2003) "Anthrax toxin" *Annual Review of Cell and Developmental Biology*, 19:45–70.

Khanna, H., and Singh, Y. (2001) "War against anthrax" Molecular Medicine 7(12):795–796.

"An Open Letter to Elias Zerhouni" *Science*, 4 March 2005.

Shane, S. (1 March 2005) "US germ-research policy is protested by 758 scientists" *The New York Times*, p A14.

Common Questions

1. Using the information you received in lecture and from the above papers, describe in detail the mechanism of how EF, LF, and PA cooperate to infect the cell.
2. Using what you have learned about the mechanism in the background and in the assigned readings, make a list of all of the possible ways that this mechanism could be disrupted, i.e., for potential drug targets.
3. Summarize the concerns of the 758 scientists who wrote a letter to Elias Zerhouni, the head of the NIH.

Interest Group Readings and Questions

Biochemists

Students assigned to the biochemists' group should read the papers listed below and then address the following questions in your oral presentation to the class.

Papers

1. Mourez, M., Kane, R.S., Mogridge, J., Metallo, S., Deschatelets, P., Sellman, B.R., Whitesides, G.M., and Collier, R.J. (2001) "Designing a polyvalent inhibitor of anthrax toxin" *Nature Biotechnology* 19, 958–961.
2. Glick, M., et al., (2002) "Pinpointing anthrax toxin inhibitors" *Nature Biotechnology* 20:188–119.
3. Khanna, H., and Singh, Y. (2001) "War Against Anthrax" *Molecular Medicine* 7(12):795–796.

Questions

1. John Collier and his colleagues at Harvard University screened a library of dodecapeptides with random sequences with the goal of isolating peptides that interfered with the binding of the EF/LF ligand to the PA_{63} heptamer. How did the researchers select for peptides that bound solely to the PA_{63} heptamer? The investigators next carried out an ELISA in order to confirm the specificity of binding of the dodecapeptides. The results are shown in Figure 2B of Reference (1) above. Each of the dodecapeptides was assessed for its ability to bind the PA_{63} heptamer, intact PA, LF_N, or some combination. LF_N is the amino-terminal portion (255 amino acid residues) of the LF protein which contains the binding site for PA_{63}. Interpret the binding data shown in Figure 2A in Reference (1) and describe the binding characteristics of each dodecapeptide. What conclusions can you make based on these binding characteristics?
2. Where is the PA_{63}-binding site for P1 and P2? Describe the molecular modeling carried out by Glick et al. in an attempt to map the anthrax inhibitor binding site. What are the important intermolecular interactions?
3. The investigators synthesized pure P1 and added it, along with PA_{63} and LF_N, to Chinese hamster ovary (CHO) cells. What were the results of this binding compared to a control peptide? What was the IC_{50} value of the P1 binding?
4. The investigators concluded that P1 would be an unsuitable inhibitor of endotoxin delivery into cells. Explain this conclusion.
5. To increase the effectiveness of P1 as an inhibitor for endotoxin delivery to macrophages, the investigators synthesized a polyvalent molecule that contained multiple copies of the P1 ligand. They and others have found that polyvalency can increase the efficiency of ligand binding to multiple receptor sites.
    a. Summarize how the inhibitor was prepared.
    b. Following synthesis, the investigators carried out native (i.e., no SDS was present) polyacrylamide gel electrophoresis and demonstrated that the polyvalent inhibitor (PVI) bound to purified PA_{63}. Draw a diagram of the PAGE results. Show four lanes: (i) PVI alone, (ii) acrylamide backbone alone (iii) PVI + PA_{63}, and (iv) acrylamide backbone + PA_{63}. What information was obtained from the gel?
6. Next, the ability of PVI to inhibit the binding of radiolabeled LF_N to PA_{63} on CHO cells was measured. The results are shown in Figure 3A of Reference (1). What are the IC_{50} values of LF_N, PVI,

and the backbone compared to PI? What is the increase in inhibitory activity for PVI as compared to PI?

7. Lastly, the investigators wished to determine whether PVI was able to inhibit the biological action of the anthrax toxin. They synthesized a fusion protein whose N-terminal was LF_N and whose C terminal was the diphtheria toxin A chain. The fusion protein was termed LF_NDTA. This protein binds to the PA_{63} heptameric complex bound to the extracellular surface and is subsequently endocytosed in a manner similar to that of the PA_{63} heptamer-EF/LF complex. Following insertion of the PA_{63} heptameric pore in the membrane of the endosome, DTA enters the cytosol and catalyzes the ADP-ribosylation of elongation factor-2 (EF-2), which inhibits protein synthesis. The rate of protein synthesis can be measured experimentally by measuring the incorporation of [^{3}H]-leucine into newly synthesized proteins. CHO cells were incubated with PA and LF_NDTA. At the same time, various amounts of LF_N, PVI, backbone, or PI was added. This type of experiment is a "competition" experiment to ascertain whether these four compounds have the ability to compete with LF_NDTA for binding to the PA_{63} heptamer. The results are shown in Figure 3B of Reference (1). Which compound(s) can inhibit the binding of LF_NDTA to PA? What is the significance of these results? Would PVI serve as a more effective inhibitor *in vivo* than PI alone? Explain.
8. What animal experiments did Collier et al. perform to test the effectiveness of PVI?
9. In a separate study (Sellman, B.R., Mourez, M., and Collier, R.J. (2001) *Science* 292 695–697), Collier and his colleagues carried out an experiment in which the PA gene was modified so that a mutant protein was produced in which the $2\beta_2$-$2\beta_3$ loops that come together to form the 14-strand β-barrel were deleted. What is the result if this mutant PA and wild type PA (along with LF_N-DTA) are added to macrophages in culture? Could the mutant PA serve as a drug to treat anthrax? Explain.

Questions for class discussion following the biochemists' presentation

Studies such as the one carried out by Collier and colleagues and Glick and colleagues have as their ultimate goal the development of either therapeutic treatments or preventative measures against anthrax. Are the reagents described in these papers effective treatments against anthrax? What concerns would you have about using these reagents?

Immunologists

Students assigned to the immunologists' group should read the papers listed below, then use information contained in the papers, class lecture, and your textbook (and any other additional resources you deem appropriate) to address the following questions in your oral presentation to the class.

Papers

1. Leppla, S. H., Robbins, J. B., Schneerson, R., and Shiloach, J. (2002) "Development of an improved vaccine for anthrax" *Journal Of Clinical Investigation* 109:141–144.
2. Singh, Y., Ivins, B.E., and Leppla, S.H. (1998) "Study of immunization against anthrax with the purified recombinant protective antigen of *Bacillus anthracis" Infection and Immunity*, 66(7):3447–8.
3. Rosovitz, M.J., Schuck, P., Varughese, M., Chopra, A.P., Mehra, V., Singh, Y., McGinnis, L. M., and Leppla, S.H. (2003) "Alanine-scanning mutations in Domain 4 of anthrax toxin protective antigen reveal residues important for binding to the cellular receptor and to a neutralizing monoclonal antibody" *Journal Of Biological Chemistry*. 278(33):30936-30944.

4. Rhie, G.-E., Roehrl, M.H., Mourez, M., Collier, R.J., Mekalanos, J., and Wang, J.Y. (2003) "A dually active anthrax vaccine that confers protection against both bacilli and toxins" *Proceedings of the National Academy of Sciences*. 100(19):10925–10930.

Questions

1. Antibiotic therapy is currently being used to treat anthrax infections, but prevention of an infection would be much preferred. A group of researchers is working on developing an improved vaccine for anthrax. To that end, it's important to understand the differences among different strains of anthrax. What are the virulent factors of anthrax? Can all of these virulent factors produce immunity? What are the characteristics of the Sterne strain and the Pasteur strain of anthrax bacteria? Which strain is preferred for producing a live, attenuated vaccine? Explain your answer.
2. Describe the AVA human vaccine (newly renamed BioThrax). How is it prepared? Is it effective? Why is it not the ideal vaccine? What questions do Leppla et al. raise concerning this vaccine?
3. Singh et al. carried out experiments designed to improve upon the AVA vaccine. They used a mutant PA and tested it in guinea pigs. Describe their experiments and summarize their results. What is the advantage of using mutant PA over wild type PA?
4. Describe the four domains of PA as illustrated in Figure 2 in Leppla et al. Leppla et al. have hypothesized that vaccine-induced immunity depends on two monoclonal antibodies that bind to two critical sites on PA. Explain.
5. One strategy of treating a disease is to challenge experimental animals with an antigen, collect the serum containing the antibodies to that antigen, and administer either the serum or the purified antibodies to a second organism to treat disease. Why is this strategy effective? What are the advantages of using this strategy instead of a vaccination strategy?
6. Antibody therapy, as described in Question 5, is what Leppla et al. had in mind when they set out to isolate mouse monoclonal antibodies directed against PA. They isolated a mouse monoclonal antibody named 14B7 that they discovered to be "toxin-neutralizing." How does 14B7 act as a toxin neutralizer?
7. How did Rosovitz et al. map the cellular binding site on PA? Describe the MTT dye assay used to test mutants in the RAW264.7 cell line. (A more sensitive assay employed CHO-CL6 cells with the FP59 fusion protein which yielded similar, but more sensitive results. A study with a furin-deficient cell line confirmed PA binding.) The data are shown in Table 2 of the Rosovitz paper. Which amino acid substitutions resulted in the greatest loss of toxicity of the mutant PA? Summarize their findings.
8. The results discussed in Question 7 indicate that there are amino acids in the loop of Domain 4 that are critical for PA binding to the receptor. The mouse monoclonal antibody 14B7 has been shown to block binding of PA to its receptor; therefore it's possible that the region mapped as described in Question 7 is also the binding region for 14B7, and if there are mutations in this binding region, 14B7 would no longer be able to bind. Was this hypothesis correct? Were the residues you identified in Question 7 also important in 14B7 binding, i.e., is the PA cellular binding site the same as the site where 14B7 binds? Would 14B7 alone be an effective therapeutic treatment?
9. How did Rhie et al. enhance the immunogenicity of PA? Summarize their findings.

Questions for class discussion following the immunologists' presentation

To prepare for the immunologists' presentation, all students should read the papers and prepare answers to the questions listed below.

Papers

1. Lipton, E. (2004) "Doubts are raised over push to supply anthrax vaccine" *The New York Times* (Dec 11th), p A1.
2. Fraser, C.M., and Dando, M.R. (2001) "Genomics and future biological weapons: the need for preventive action by the biomedical community" *Nature Genetics* 29:253–256.

Questions

1. What do you think of Project BioShield? Is this program necessary? If it is put into place, will it be effective?
2. Should military personnel be vaccinated against anthrax? Should this be a requirement? Should the entire U.S. population be vaccinated against anthrax?
3. In their conclusion in a Commentary written shortly after the September 11 attacks, Fraser and Dando state that "…we need to develop and fund specific research programs aimed at addressing the threat of biological weapons, rather than hoping that relevant progress will be made as a consequence of research activities in more benign areas." Do you agree with this statement?

Microbiologists

Students assigned to the microbiologists' group should read the papers listed below, then use the information from the papers, class lecture, and your textbook (and any other additional resources you deem appropriate) to address the following questions in your oral presentation for class.

Papers

1. Athamna, A., Massalha, M., Athamna, M., Nura, A., Medlej, B., Ofek, I., Bast, D., and Rubinstein, E. (2004) "In vitro susceptibility of Bacillus anthracis to various antibacterial agents and their time-kill activity" *Journal of Antimicrobial Chemotherapy* 53:247–251.
2. Brookmeyer, R., Johnson, E., and Bollinger, R. (2003) "Modeling the optimum duration of antibiotic prophylaxis in an anthrax outbreak" *Proceedings of the National Academy of Sciences* 100(17):10129–10132.
3. Hart, C.A., and Beeching, N. J. (2001) "Prophylactic treatment of anthrax with antibiotics" *Biomedical Journal* 323:1017–1018.
4. Vastag, B. (2002) "'Cipromania' and 'Superclean' homes are now increasing antibiotic resistance" *Journal of the American Medical Association* 288:947–948.

Questions

1. Explain in general how antibiotics work to eradicate disease-causing organisms from the body. Why are antibiotics ineffective against viruses?
2. Do some research on your own and determine the mechanism of action of the following classes of antibiotics: (a) fluoroquinolones, (b) β-lactams, (c) macrolides, (d) tetracyclines (e) quinupristin/dalfopristin (f) rifampicin, and (g) chloramphenicol.

3. Athamna et al. tested the ability of the antibiotic agents above to treat anthrax. Which antibiotics were the most effective? Summarize their results.
4. Brookmeyer et al. developed a model to determine how long antibiotics should be taken prophylactically after an anthrax attack. You don't need to explain the complex mathematics, but you should be able to explain the results shown in the two figures and the table in the paper. What is the optimum duration of antibiotic prophylaxis? What assumptions did the authors make in developing their model?
5. In an Oct. 19, 2001, interview with Professor Stuart Levy, a microbiologist at Tufts University, Ira Flatow, the host of the NPR program *Science Friday*, asked why physicians who treated victims of anthrax infection (or those who had merely been exposed) prescribed ciprofloxacin rather than penicillin. Why were patients given Cipro instead of penicillin? Was this a prudent decision? Explain.
6. Why are some bacteria resistant to the effects of penicillin? How would you genetically engineer a strain of anthrax to be resistant to penicillin?
7. What are some of the dangers of using potent antibiotics like Ciprofloxacin prophylactically on a large segment of the population, according to Hart and Beeching, and Vastag?

Questions for class discussion following the microbiologists' presentation

1. Patients who were worried about contracting anthrax pleaded with their physicians to prescribe ciprofloxacin, whether or not they had been exposed to the bacterium. Some physicians actually complied with these requests. What are the ramifications of large-scale antibiotic prescriptions to individuals who may or may not have been exposed to the disease? How does a physician balance the needs and desires of an individual patient with the needs of the society as a whole? What problems might develop if these patients did not fulfill the entire course of antibiotic treatment after the "scare" was over?
2. "The diversion of research funds from projects of high public-health importance to projects of high biodefense but low public-health importance represents a misdirection of NIH priorities and a crisis for NIH-supported microbiological research" write the 758 scientists in a letter to Elias Zerhouni. Do you agree or disagree with the sentiments expressed in this letter?