Comment

Biodefense versus bioterrorism

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We always knew, didn't we, that he was probably one of our kind. When the deadly envelopes laced with anthrax spores claimed their first of five eventual victims - Robert Stevens, a 63-year-old photo editor for the Florida-based tabloid 'newspaper' *The Sun* - just a few weeks after the 11 September 2001 terrorist attacks on New York and Washington, most scientists figured that the killer was connected with the US biowarfare program, and was almost certainly a scientist. Using anthrax as a weapon required just too much specialized knowledge for the low-tech terror groups we were used to dealing with; it was simpler to imagine that the powder came from someone with access to a bioweapons stock and knowledge of how to handle it. Now, with the recent suicide of a microbiologist who worked at the US Army Medical Research Institute of Infectious Diseases at Fort Detrick, Maryland, and the partial release of evidence from the Federal Bureau of Investigation (FBI) that indicates he was probably the person responsible, our worst fears have been realized. Pogo, Walt Kelly's philosophical cartoon possum, was right: "We have met the enemy, and he is us."

In the past few days, new details of how this case was finally solved after almost seven years have come to light, and what they reveal is the critical role of genomics in identifying the source of the anthrax. In fact, many of the techniques did not even exist at the time the anthrax letters were mailed. As he prepared his poisonous envelopes, the murderer could not have imagined that their origin would eventually be revealed by technology beyond his/her ken. As Hamlet put it: "For murder, though it hath no tongue, will speak with most miraculous organ." In this case, the miraculous organ of genomics.

The best account I've read of this fascinating piece of microbial forensics is the front-page story by Nicholas Wade in the 21 August edition of the *New York Times* [http://www.nytimes.com/2008/08/21/science/21anthrax.html?partner=rssnyt&emc=rss]. I'm drawing heavily on this superb article as I try to summarize what happened.

Paul Keim, a biologist at Northern Arizona University in Flagstaff, was able to determine from an anthrax sample taken from Robert Stevens' corpse that the strain used was the virulent Ames strain. To identify its source, the FBI hired a team at The Institute for Genome Research (TIGR), where the first microbial genomes had been sequenced in the dawn of the genomics era, to determine the complete DNA sequence of the lethal strain. This they did, in about four months (it could be done in days today). When the sequence was compared with that from a culture of Ames anthrax maintained at Porton Down in Britain, the UK government's research establishment for defense against biological weapons, several differences were found between the anthrax genome taken from Stevens and the genome of the Porton Down strain. The Ames strain originated from a cow that died of anthrax in Texas in 1981, so the next step was for TIGR to sequence that ancestral strain, so that a phylogenetic tree of anthrax substrains could be constructed. Unfortunately, the bioterror strain turned out to be virtually identical to the original Ames strain, so it looked as if its source could not be identified by sequencing.

What finally cracked the case was an inspired bit of old-fashioned microbiology. An army scientist at Fort Detrick noticed that cultures of the bioterror strain were not uniform: one of the colonies had an altered morphology, suggesting that a small percentage of the cells in the sample harbored mutations. The odd colony was grown up and TIGR sequenced its genome; sure enough, there was a small but significant change. Eventually, a total of eight morphological variants were identified in the bioterror strain, and all were sequenced. The lethal strain now had a genetic fingerprint, the first ever obtained for a microbe: the pattern of genetic changes in the 1% of spores in the sample that were different from the other 99%. It turned out much later that the reason there were so many variations was that the flask that contained the anthrax strain used in the attacks held the results of 35 separate preparations of anthrax, giving the strain ample opportunity to develop mutations. It

was soon determined that all of the anthrax letters contained bacteria from the same source.

The FBI was now able to compare this signature pattern of variations, the bacterial version of the single nucleotide polymorphisms (SNPs) that are used in DNA fingerprinting of people, to those of anthrax samples obtained from laboratories around the world. By 2006 it was clear that the source of the bioterror strain was a flask in the laboratory of Bruce Ivins, a microbiologist at Fort Detrick with a history of mental instability. But it took two more years before the investigators succeeded in eliminating other scientists who might have had access to the flask as possible suspects. As the FBI began to focus its inquiry on Ivins, he took his own life.

Genomics cannot prove that this man sent the anthrax letters in 2001, but its success in identifying the source of the strain marks a landmark in scientific detective work. As whole organism sequencing becomes even cheaper and faster, we may see the day when the genetic signature of microbes found at the scene of the crime, or on the clothing of a suspect, become as damning evidence as the suspect's own DNA. And the new heroes of *Crime Scene Investigation (CSI)* could be microbial genome biologists.

Ivins was not a bioweapons scientist. His job at Fort Detrick was to find an effective vaccine against anthrax strains that had been turned into a bioweapon. It has been speculated that his motive in sending the anthrax letters - if he was the person who did so - may have been to increase demand for the vaccine he developed. Another possibility is that, as he is known to have believed that the United States was taking the possibility of bioterrorism too lightly, he may have carried out the attacks to raise awareness of the danger and generate political support for increased focus on biodefense. That would explain why Senate offices were among the targets.

If that was the tactic, it had unintended consequences: immediately after the anthrax attacks, White House officials repeatedly pressured FBI director Robert Mueller to prove that they were by Al-Qaeda, but the FBI knew very early in the case that the anthrax used was a version requiring sophisticated equipment and was unlikely to have been produced by a low-tech terrorist organization. Nevertheless, President Bush and Vice President Cheney both speculated publicly about the possibility of a link between the anthrax attacks and Al-Qaeda. The *Wall Street Journal* published an editorial stating that Al-Qaeda perpetrated the mailings and that Iraq was the source of the anthrax, thereby displaying their remarkable ignorance of Middle Eastern affairs: a secular state like Iraq, ruled by a secular dictator, would be the last place Al-Qaeda would go for help. A few days later, Senator John McCain, who will soon be the Republican contender for the presidency of the United States, displayed equal ignorance of foreign affairs by suggesting on the *David Letterman Show* that the anthrax may have come from Iraq. Of course, this possibility was one of the many falsehoods that the Bush administration used as the rationale for their later invasion of that country.

In another sense, however, the attacks may have had the intended consequences, because shortly after they occurred, Congress voted massive increases in spending for biodefense research, to the tune of more than a billion dollars earmarked for the National Institute of Allergy and Infectious Diseases (NIAID). Since the recent revelation that the source of the anthrax was our own army research program and that one possible motive for the killings was to provoke just such an increase in support, there has been criticism that the government overreacted and is investing far too much money in research aimed at counteracting bioterrorism and far too little to deal with less sophisticated forms of terror attacks.

I think this criticism is half right: we are paying too little attention to the possibility of simple methods of achieving widespread destruction. In our focus on foreign terror groups and high-tech weaponry, we seem to have forgotten that, until the events of 11 September the deadliest terror attack on American soil was perpetrated by a clean-cut former US soldier, who used a truck full of diesel fuel and fertilizer to blow up the Oklahoma City Federal Building. Chemical weapons are cheap and easy to manufacture and simple to deliver. The same is true of spreading radioactive material. Low-tech terror offers far more choices of means and plenty of technical and operational options.

But I don't think we are spending too much money on biodefense, even though I believe the possibility of more attacks like the anthrax letters is remote. I think we need every dollar we're spending, but not to protect us from bioterrorism. It's to protect us from infectious diseases.

More than 25% of the world's deaths are due to infectious disease, and there is no effective means of prevention or treatment for many of the most deadly agents, such as Ebola, Marburg and the other hemorrhagic fever viruses. HIV/AIDS is controllable with expensive drug cocktails in the developed world but has so far resisted all attempts at effective vaccine development. Multidrug-resistant tuberculosis is becoming endemic in some parts of the world and is a potential public-health catastrophe should it spread widely. The devastating economic consequences of the SARS virus outbreak point up how vulnerable the world's economies are to heath-related disruption. Avian flu seems to have quietened down but is still out there somewhere, waiting to learn how to jump the species barrier. Methicillin-resistant *Staphylococcus aureus* and other so-called 'superbugs' are threatening to turn every hospitalization into a game of Russian roulette. There have been hardly any new classes of antibiotics developed in the last quarter century, and resistance to the ones we have is on the rise. Vaccine development is technically difficult and economically risky;

antibiotic development has largely been ignored by the major pharmaceutical companies in recent years and is now in the hands of a few underfunded, and undermanned, biotechnology companies. We are dangerously close to returning to the situation of my mother's childhood, 90 years ago, when virtually every cut or scrape was potentially life-threatening, and more people perished from infectious disease during wars than died on the battlefields.

But exactly the same research that is needed to combat low-probability bioterror attacks is needed to combat high-probability natural infectious agents. Every project that NIAID funds to protect soldiers and civilians from manmade bioweapons could provide a breakthrough in the fight against AIDS, or tuberculosis, or the flu, or any of the thousand other natural shocks that flesh is heir to. And as such research is not going on to the necessary degree in the private sector, it must be publicly funded if we are to have a hope of success.

In a world where irony is commonplace, this may be one of the great ironies of all: that the anthrax attacks of 2001 may have prodded us into much-needed research on biodefense, even though bioterrorism is a remote possibility. But that's the great thing about effective agents of defense: they don't care where the attack is coming from - whether it's from nature or, God forbid, from one of us.