Comment

Winter, plague and pestilence

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> "From winter, plague and pestilence, good Lord, deliver us."
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> *Sixteenth century prayer, attributed to Thomas Nashe*

Germ warfare. A new terror from an old idea - almost as old, perhaps, as war itself. During the Middle Ages armies besieging a city would sometimes hurl the corpses of plague victims over the ramparts in an attempt to infect the population within the walls. In 1864, during the American Civil War, a group of Confederate spies (today they would be called terrorists) based in Canada attempted to spread yellow fever through the cities of the North via the clothing of the disease's victims. An outbreak of human anthrax occurred in Sverdlovsk, Union of Soviet Socialists Republic (now Ekaterinburg, Russia) in April 1979. Soviet officials attributed this to consumption of contaminated meat, but Western governments believed it resulted from inhalation of spores accidentally released from a nearby biological weapons research facility. Almost twenty years later, Paul Jackson of the Los Alamos National Laboratory and Paul Keim at Northern Arizona University, together with associates from the U.S. and Russia, obtained tissue samples from the corpses of 11 victims and applied new methods they had developed for efficiently extracting high-quality total DNA from these samples. Extracted DNA was analyzed using PCR to determine whether it contained sequences specific to *Bacillus anthracis*. Results demonstrated that the entire complement of *B. anthracis* toxin and capsular antigen genes required for pathogenicity were present in tissues from each of the victims, so the sequences did not come from a vaccine strain of *B. anthracis*. PCR analysis using primers that detect the vrrA gene variable region on the *B. anthracis* genome demonstrated that at least four of the five known strain categories defined by this region were present in the tissue samples: but only one category is found in a single *B. anthracis* strain. The conclusions are obvious, and chilling. (For a complete account, see Jackson *et al., Proc Natl Acad Sci USA* 1998, **95:**1224-1229.)

Now a new word has joined the lamentable lexicon of human horrors: bioterrorism. The sight of white powder on envelopes is enough to send people racing to their doctors. Hordes of Americans are stockpiling an antibiotic they had never heard of (Cipro, the trade name for Bayer's ciprofloxacin hydrochloride, for the treatment of inhaled anthrax). *B. anthracis*, the causative agent of cutaneous, gastrointestinal and inhaled anthrax, is almost an ideal bioweapon. It is a spore former, so it can survive desiccated for months to years. It is very deadly in aerosolized form, killing about 90% of those who inhale it. Research on anthrax as a biological weapon began more than 80 years ago. Today, at least 17 nations are believed to have offensive biological weapons programs, although it is uncertain how many are working with anthrax; Iraq, for one, has publicly acknowledged producing it. The manufacture of a lethal anthrax aerosol is probably beyond the capacity of individuals or groups without access to advanced biotechnology; but autonomous groups with substantial funding and contacts with states that sponsor terrorism may be able to acquire the required materials and expertise. One terrorist group, Aum Shinrikyo, responsible for the release of the nerve gas sarin in a subway station in Tokyo, Japan, in 1995, dispersed aerosols of anthrax and botulism throughout Tokyo on at least eight occasions. The attacks failed to produce any illness, which is comforting to know, but it is not comforting to know that the reasons for this failure are still unclear.

An anthrax aerosol would be odorless and invisible following release and would have the potential to travel many kilometers before disseminating. In 1970, a World Health Organization (WHO) expert committee estimated that casualties following the theoretical aircraft release of 50 kilograms of anthrax over a developed urban population of 5 million would be about 250,000, of whom 100,000 would probably die without treatment. A 1993 report by the US Congressional Office of Technology Assessment estimated that between 130,000 and 3 million deaths could follow the aerosolized release of 100 kilograms of anthrax spores upwind of

Washington DC, a mortality comparable to that expected from a nuclear weapon. An economic model developed by the Centers for Disease Control and Prevention (CDC) suggested a cost of $26.2 billion per 100,000 persons exposed.

If all this is frightening... well, that is the objective of terrorism. But terror itself can harm much more than anything the terrorists can do. Terror can cause us to surrender our freedoms in pursuit of a false sense of security, and to make decisions based on our fears rather than on facts. Here are the facts, taken in large part from a report by the Working Group on Civilian Biodefense from the American Medical Association, published in the Association's *Journal* on May 12, 1999.

The name *Bacillus anthracis* derives from the Greek word for coal, anthrakis, because the disease causes black, coal-like skin lesions. *B. anthracis* is an aerobic, Gram-positive, spore-forming, nonmotile bacillus species. The nonflagellated vegetative cell is large (1-8 μm in length, 1-1.5 μm in breadth). Anthrax spores germinate when they enter an environment rich in amino acids, nucleosides, and glucose, such as that found in the blood or tissues of an animal or human host. Vegetative anthrax bacilli will form spores only after local nutrients are exhausted, as happens, for example, when anthrax-infected body fluids are exposed to ambient air. Full virulence requires the presence of both an antiphagocytic capsule and three toxin components: protective antigen, lethal factor, and edema factor. Vegetative bacteria have poor survival outside an animal or human host; colony counts decline to undetectable within 24 hours following inoculation into water. The spore can survive for decades. Inhalational anthrax follows deposition of spore-bearing particles of 1 to 5 μm into alveolar spaces. Macrophages ingest the spores, some of which undergo lysis and destruction. Surviving spores are transported via the lymphatic system to mediastinal lymph nodes, where germination may occur up to 60 days later. (The process responsible for the delayed transformation of spores to vegetative cells is poorly understood but well documented. In Sverdlovsk, cases occurred from 2 to 43 days after exposure.) Once germination occurs, disease follows rapidly. Replicating bacteria release toxins leading to hemorrhage, edema, and necrosis. In experimental animals, once toxin production has reached a critical threshold, death occurs even if sterility of the bloodstream is achieved with antibiotics. On the basis of primate data, it has been estimated that for humans the LD 50 (lethal dose sufficient to kill 50% of persons exposed to it) is 2,500 to 55,000 inhaled anthrax spores. Mortality rate is estimated at 90% after first symptoms appear.

But - and here's the crucial point - those mortality figures are derived from very old studies, long before there were very effective antibiotics available. And there is a superb one available now: Cipro, the first of the fluoroquinolone antibiotics. These compounds do not work in a penicillin-like fashion by disrupting cell-wall synthesis; they are DNA gyrase inhibitors, attacking an enzyme unique to bacterial replicative machinery. Fluoroquinolones are broad-spectrum and relatively free of side-effects. They are very effective against urinary tract infections, which are the leading bacterial infections in most developed countries. And they are very effective against *B. anthracis*, in part because, as far as is known, a resistant bioweapons-grade strain has not been developed.

But if people everywhere now start hoarding Cipro and taking it for every winter cold or flu symptom that comes along, or prophylactically, which is probably useless, we may not have this situation for long. Resistance arises from just such usage, and resistance travels throughout the bacterial world with great speed. The complete genome sequence of the bacterium *Yersinia pestis*, the causative agent of the bubonic plague, which was just reported (Parkhill *et al., Nature* 2001, **413:**523-527), reveals that this little microbe, which in the fourteenth century killed about a third of the population of Europe, contains many genes that seem to have been acquired from other bacteria and viruses (including adhesins, secretion systems and insecticidal toxins). Pathogens are like that: they constantly acquire resistance genes and virulence factors. Indiscriminate use of antibiotics just plays into their hands.

Besides, anthrax isn't the only threat. *Y. pestis* has been tinkered with in germ warfare factories too. So have *Clostridium botulinum*, and the tetanus bacillus. Viral diseases, like Ebola, Marburg and smallpox, which are contagious, are potentially a greater hazard. And yet, individual acts of terrorism do not a widespread terrorist assault make. It is very difficult to make and deliver bioweapons on a wide scale, and they have the nasty habit of not respecting borders, or politics, or religions. Governments are unlikely to use them for that reason, and terrorist cells are probably unable to mount them as a large-scale threat. The danger, then, is in our overreaction. Vigilance is an appropriate response; terror is not.

Research is also an appropriate response. We need more microbiologists, and more work by pharmaceutical companies on microbial diseases, an area they largely abandoned thirty years ago. We need funding for innovative methods of rapid diagnosis, strain typing, and antimicrobial treatment. More microbial genome sequences, especially of different strains of pathogenic organisms, would also be very useful. And we need something else: condemnation, and swift justice, for those who create or deploy such weapons. Nations, like individuals, have the right to defend themselves. But bioweapons are different: because they do not respect borders or uniforms, they are not defensive weapons. Their only use is far from home, in an offensive strike. I believe that anyone who creates or helps to create such a weapon is a traitor to science and should be brought to trial in an international court of law for crimes against humanity.