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Living Nightmares: Biological Threats Enabled by Molecular Biology

This chapter draws on a 1997 summer study by JASON¹ that I led on the threat posed by the development and use of biological agents. Rather than address the myriad prob-

Thanks to the JASON Group, and especially to the participants in the 1997 Summer Study described in footnote 1 below, for their contributions to this chapter. Thanks also to Princeton University's Lynn Enquist (also editor, *Journal of Virology*) for his insight, encyclopedic knowledge, and patient tutorials in virology. Finally, thanks to Sidney Drell for inspiring me to think hard, for reawakening my latent activism, and for giving me the opportunity to do something about it.

1. This study was held during June and July of 1997. The JASONS are a collection of primarily academic scientists who dedicate a portion of their time to addressing problems of national interest, consulting for various US government agencies. According to their charter (JASON Charter, as amended November 13, 1991, Mitre Corp., McLean, VA):

JASON exists to enable scientists to contribute to the enhancement of national security and to the public benefit by working as individuals or in groups on problems of impor-

lems posed by biological agents in general—a daunting and hopeless task—the JASON study focused instead, and from the outset, on one issue. Specifically, we took a hard look at what the near-term future of biological warfare held, based on how recent advances in the life sciences have changed the nature and scope of that threat. In brief, we concluded that progress in biomedical science inevitably has a dark side, and potentiates the development of an entirely new class of weapons of mass destruction (WMD): genetically engineered pathogens. The danger of such next-generation biological weapons (BW) in the twenty-first century is quite real, and they pose

tance supported by government organizations

Formed during the Cold War era, the JASON group has been particularly active in confronting the nuclear threat. From the outset, its membership has included prominent scientists with backgrounds mainly in physics and mathematics. With the wind-down of the Cold War, however, the composition of the group shifted in keeping with the changing focus, and has grown today to include experts in computer science, electrical engineering, molecular biology, and other fields. In addition to the diversity of science represented by the group, individual JASON members have always been distinguished by their broad, cross-disciplinary interests. The 1997 Summer Study was carried out by me and the following fourteen individuals: Curtis Callan (Princeton University), J. Mike Cornwall (University of California, Los Angeles), William Dally (Stanford University), Freeman Dyson (Institute for Advanced Study), Norval Fortson (University of Washington), Gerald Joyce (Scripps Research Institute), H. Jeff Kimble (California Institute of Technology), Steven Koonin (California Institute of Technology), Claire Max (Lawrence Livermore National Labs), Thomas Prince (California Institute of Technology), Oscar Rothaus (Cornell University), Roy Schwitters (University of Texas), Peter Weinberger (Renaissance Technology), and W. Hugh Woodin (University of California, Berkeley). The study group included an MD/PhD as well as a biologist, in addition to one or more physicists, astronomers, mathematicians, computer scientists, and engineers. Many of the same individuals participated that summer in a study of the Human Genome Project (S. E. Koonin, “An Independent Perspective on the Human Genome Project,” 279 *Science* (1998), pp. 36–37), undertaken on behalf of the Department of Energy. While assessing work at the forefront of life science, it seemed natural to contemplate at the same time the dangers of what has been termed “black biology.” In this chapter, I write strictly as an individual, and not in any capacity on behalf of JASON, but it would be inappropriate not to acknowledge their vital role in shaping this presentation.

extraordinary challenges for detection, mitigation, and remediation.²

Prescience?

The JASON study proved to be rather timely. Shortly after our report was briefed, a popular account of bioterrorism with a genetically engineered pathogen was published, eventually making it onto the bestseller list: Richard Preston's *The Cobra Event*.³ Although its plot was fictional—right down to the impossible supervirus it described—many of the supporting details in the novel were well researched and scientifically accurate. No question about it, this was scary stuff, a real eye-opener. Preston's book was reportedly read by President Clinton,⁴ who was motivated to confer directly with a number of prominent biologists and biotechnologists about the credibility of Preston's scenario. Two Presidential Decision Directives have since emerged that address aspects of chemical, biological, and cyber threats, along with other security issues (PDDs 62 and 63). During 1997–98, prospects for some form of BW attack, involving either conventional or genetically engineered agents, were reported extensively by the news media.⁵ Even the *Journal of the American Medical Association* devoted an entire issue to biological warfare concerns.⁶

2. The color figures that accompanied my presentation at the November 16–18, 1998, Hoover Institution conference on biological and chemical weapons have been rendered in black and white for this chapter.

3. Richard Preston, *The Cobra Event* (New York: Random House, 1997).

4. Judith Miller and William J. Broad, "Exercise Finds US Unable to Handle Germ War Threat," *New York Times* (Apr. 26, 1998), p. 1.

5. Coverage included television network specials on ABC (*PrimeTime Live*, Feb. and Jul. 1998), TBS (*The Coming Plague*, Apr. 98), and PBS (*Frontline*, Oct. 1998), plus a series of articles in the *New York Times*.

6. 278 *Journal of the American Medical Association* (no. 5) (Aug. 6, 1997), pp. 347–446.

This level of media attention undoubtedly aroused public interest in biological warfare—but was it alarmist hype, or largely warranted? On balance, I am inclined toward the latter view, for the following reason. Modern bioscience has led to the development of many powerful tools for manipulating genes. Such tools hold the key to revolutionary medical advances, among them gene therapy and the eventual abolition of fatal diseases such as cancer. But they make equally possible the creation of entirely new WMD, endowed with unprecedented power to destroy. It seems likely that such weapons will eventually come to exist, simply because of the lamentable ease with which they may be constructed. In contrast to nuclear weapons, BW do not require rare materials, such as enriched uranium or plutonium. They do not require rare finances: development and production are comparatively inexpensive. They do not require rare knowledge: most of the techniques involved are straightforward, well-documented, and in the public domain. Today, thousands of biologists worldwide possess the requisite skills, and more are trained every day (most often at US universities). Finally, they do not require rare infrastructure; some BW can be produced by small terrorist groups almost as easily as through national biological warfare programs. Inevitably, someone, somewhere, sometime seems bound to try something. So, for better or worse, genomics will change our world (see figure 2-1). It would be tragic if it took the biological equivalent of Hiroshima to muster our response. Like it or not, we need to begin certain preparations now.

Dimensions of the Threat

What can we anticipate? It seems reasonable to draw an analogy with the way that today's nuclear threat developed. In the pre-World War II era, the largest conventional bombs carried up to ~20 tons of TNT explosive. With the advent of the atomic bomb in 1945, explosions unleashed a 1,000-fold more power. The fission

Living nightmares: Next-generation BW threats

- Biotechnology is *very* powerful. It is also relatively inexpensive, and does not require special infrastructure. It is based on public knowledge. It is becoming ubiquitous.
- Rapid advances in molecular biology make it necessary to contemplate new BW threats.
- Genomics will change the world.

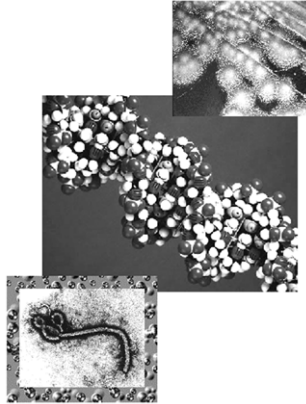


Figure 2-1

bombs dropped on Hiroshima (“Little Boy,” August 6, 1945) and Nagasaki (“Fat Man,” Aug. 9, 1945) released the equivalent of ~12 and ~23 kilotons of TNT, respectively. Within a decade, fusion-based devices were developed that made fission weapons seem puny by comparison. By 1954, H-bomb detonations in Operation Castle on the Bikini atoll released the equivalent of ~15 megatons of TNT—1,000-fold more than A-bombs, and 1,000,000-fold more than conventional weapons. By the height of the Cold War, the former USSR was testing thermonuclear weapons equivalent to 50 megatons of TNT. Each major breakthrough in weapons development produced roughly three orders of magnitude greater destructiveness. The type of biological weaponry made possible by genetic engineering may conceivably produce an analogous increase in virulence over conventional biological agents, which in turn have greater destructive potential than natural outbreaks of disease. If the analogy holds, this is bad news indeed.

In truth, there is little that is “conventional” about conventional

Conventional BW agents

- Bacterial agents: anthrax, plague, tularemia, brucellosis, typhoid fever
- Rickettsial agents: typhus, Rocky Mtn. spotted fever, Q fever
- Viral agents: smallpox, influenza, yellow fever, encephalitis (various), dengue fever, chikungunga, Rift Valley fever, hemorrhagic fevers (Ebola, Marburg, Lassa)
- Toxins: botulinum toxin, staphylococcus enterotoxin, shigella toxin, aflatoxin
- Fungal agents: coccidioidomycosis
- Other: antiplant, antianimal, etc.

Figure 2-2

biological agents, which are already bad enough! These weapons comprise some of the worst scourges of mankind, such as smallpox, typhoid, typhus, anthrax, and plague. They include viral hemorrhagic fevers, such as Ebola, Lassa, and Marburg. They include potent toxins produced by microorganisms, such as botulinum toxin, aflatoxin, and shigella toxin. And the list goes on—see figure 2-2 for some examples.

In certain respects, a case can be made that anthrax is *already* a nearly perfect biological weapon in its natural form—indeed, it has long been the weapon of choice for many of the identified biological warfare programs. The anthrax bacterium, *Bacillus anthracis*, is ubiquitous and easily cultured from soil in areas that support livestock. It is readily propagated on inexpensive media. When grown appropriately, anthrax forms highly stable spores that retain their potency for decades as a dessicated powder,⁷ ideal for weaponization. A mere

7. C. Redmond, M. J. Pearce, R. J. Manchee, and B. P. Berdal, “Deadly Relic of the Great War,” 393 *Nature* (1998), pp. 747–8.

gram of spores contains thousands of lethal doses. Although deadly once contracted, anthrax is only weakly communicable, making it less prone to spread to friendly forces. Bacteria must enter directly into the lungs (*inhalation anthrax*, historically called woolsorter's disease) or into the bloodstream through a wound (*cutaneous anthrax*) to produce serious illness, although ingestion of badly contaminated meat may also produce disease (*intestinal anthrax*). When used as a biological weapon, inhalation anthrax is induced by exposure to large numbers of airborne spores. Enough spores in the lungs can produce death within a few days (the incubation period is one to six days, with a mortality rate of ~80 percent). However, the initial symptoms of pulmonary anthrax infection are fairly unremarkable (low fever, hacking cough, and weakness) and may make early diagnosis more difficult.

Anthrax is by no means the perfect bioweapon, however, for several excellent but unrelated reasons. First and foremost, it is non-trivial to target a ground population with any airborne agent, due to the many difficulties of dissemination, that is, producing just the right aerosol, adjusting for the vagaries of wind and weather, and so forth.⁸ Second, prolonged exposure to sunlight kills most anthrax spores after release. Third, the minimal lethal dose for inhalation (reported to be 5,000 to 10,000 spores) is high compared with some other biological agents. Fourth, if diagnosed and treated early, anthrax may be cured with sufficient doses of penicillin-type antibiotics. Fifth, specific vaccines can be prepared that prevent infection by known strains of anthrax.

8. The Japanese terrorist cult Aum Shinrikyo (Aum Supreme Truth) is reported to have released quantities of anthrax in repeated biological attacks during the summer of 1993—all of which failed, and partly for this reason. In 1995, after apparently abandoning their biological warfare efforts, they released sarin gas in an attack on a Tokyo subway, killing 12 people and injuring 5,000. William J. Broad, "Sowing Death: A Special Report. How Japan Germ Terror Alerted the World," *New York Times* (May 26, 1998).

Novel BW threats

- Genetically engineered pathogens are qualitatively different from conventional BW agents.
- Attributes may include one or more of the following:
 - Safer handling and deployment
 - Easier propagation and/or distribution
 - Improved ability to target the host
 - Greater transmissivity, infectivity
 - More difficulty in detection
 - Greater toxicity, more difficulty in combating
 - More (self-limiting, self-enhancing ...)

Figure 2-3

Biological Warfare Desiderata

A scientist bent on producing a biological weapon more effective than airborne anthrax might consider ways to imbue the pathogen of his/her choice with any of a variety of desirable properties. These might include one or more of the following (see figure 2-3):

1. *Safer handling and deployment.* Biological warfare agents pose direct threats to those who use them, and many deaths appear to have resulted from accidental releases of agents, not from their use as weapons. What if this “boomerang problem” could be alleviated?
2. *Easier propagation and/or distribution.* Dried bacterial spores must have the right size and surface charge to disperse properly. Conversely, normally hydrated bioagents make poor aerosols. What if one could produce a better aerosol? Better

yet, what if one didn't need an aerosol at all, but relied on a different mechanism for distribution?

3. *Improved ability to target the host.* What if an agent could be developed that specifically targeted one or another population group? Or, what if some group could be protected against infection in advance?
4. *Greater transmissivity, infectivity.* What if one could engineer a viral disease with the lethality of (say) Ebola, but with the communicability of measles?
5. *More difficulty in detection.* What if the disease was hard to diagnose? Or had never even been encountered before? Or had a long latency? Or behaved in any other cryptic way?
6. *Greater toxicity, more difficulty in combating.* What if the disease had unusually high morbidity or mortality? What if it was resistant to all known antibacterial or antiviral agents? Or defeated all existing vaccines?
7. *More (self-limiting, self-enhancing . . .).* What if some pathogen could produce a localized outbreak but then render itself harmless? Conversely, what if a pathogen could continually alter itself in such a way as to evade treatment?

Clearly, some elements of this "wish list" seem rather far away from the current state of the art. But some may be closer to hand than one imagines. Recently, it was speculated in the *Times* of London that at least one country with a biological warfare program (Israel) was working on a bioweapon to target victims on the basis of ethnic origin.⁹ Although this report seems scarcely credible (par-

9. U. Mahnaimi and M. Colvin, "Israel Planning Ethnic Bomb as Saddam Caves In" (London) *Sunday Times* (Nov. 15, 1999). The article contained the following text: "Israel is working on a biological weapon that would harm Arabs but not Jews, according to Israeli military and western intelligence sources. The weapon, targeting

ticularly in light of the history of the Holocaust and anti-Semitism in general) and was not picked up by other major media, what seems noteworthy is that the *theoretical possibility* of such a development is now taken seriously. As for some of the other attributes listed, there are plenty of existing pathogens with properties fitting the bill—and which might therefore be adapted by genetic engineering. For example, it is hard to imagine a disease more communicable, or much more virulent, than smallpox.¹⁰ In terms of being hard to combat, the AIDS virus continually mutates and develops resistance to existing antiviral agents, such as AZT. Worse, it is a retrovirus, with a unique capacity to exist stably inside our cells and elude the immune system, making it nearly impossible to destroy. Exposure to minute levels of the agent aflatoxin produces fatal liver cancer, but only after a latency of years, making it hard to pin down the causative agent. And hospitals and health organizations must now come to grips with the worldwide emergence of virulent strains of multi-drug resistant bacteria (*streptococcus*, *staphylococcus*, *gonorrhoea*, etc.) that have

victims by ethnic origin, is seen as Israel's response to Iraq's threat of chemical and biological attacks. . . . Porton Down, Britain's biological defence establishment, said last week that such weapons were theoretically possible. 'We have reached a point now where there is an obvious need for an international convention to control biological weapons,' said a spokesman."

10. Smallpox was declared eradicated by the World Health Organization (WHO) in May, 1980, and numerous frozen isolates of the virus (*Variola major*) are today maintained for the WHO by the US Centers for Disease Control (CDC) in Atlanta and by the Russian State Research Center of Virology and Biotechnology in Koltsovo. There are reports of unauthorized stocks elsewhere in the Soviet Union. The DNA sequence of *Variola* is known, and there is a controversial proposal to destroy all remaining stocks worldwide on June 30, 1999, under WHO supervision. A highly effective vaccine for smallpox has long existed, but it confers immunity for only about a decade. Therefore, adults who were vaccinated in their youth are no longer protected, and most civilian vaccination programs ceased worldwide in the 1960s and 70s. Some soldiers are still vaccinated.

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emerged in response to the widespread overuse of antibiotics, both by humans and in the agricultural sector.¹¹

There have been documented efforts to alter the properties of existing pathogens in such a way as to improve their effectiveness in biological warfare. Notable here is the work of Dr. Ken Alibek (formerly Dr. Kanatjan Alibekov, the Deputy Director of Biopreparat, the USSR's bioweapons program), who defected to the US in 1992. Alibek holds a doctorate in "industrial biotechnology," awarded in 1990 for his own contribution to the development of an anthrax strain designed specifically for weaponization purposes.¹² Alibek reports that he supervised, at one point, as many as 32,000 people in some forty separate facilities (there were reportedly some 60,000 people involved in the program overall). The size and scope of the former Soviet biological warfare program seem staggering; it represented an enterprise of a scale dwarfing worldwide efforts to sequence the human genome. The Soviet bioweapons program was carried out in violation of the 1972 Biological and Toxic Weapons Convention, to which the USSR was a signatory, and was reportedly dismantled in 1992 by order of Boris Yeltsin. However, Alibek and other experts have expressed reservations about whether biological warfare work in the former Soviet Union has actually ceased.¹³

It is not known to us, for example, whether Soviet strains of anthrax were engineered to carry a form of antibiotic resistance, nor

11. Arguably, the emergence of multidrug-resistant bacteria may pose more of an immediate threat to world health than any biological warfare agents, real or potential.

12. Richard Preston, "The Bioweaponeers," *New Yorker* (Mar. 9, 1998), pp. 52–65.

13. Ken Alibek, "Terrorist and Intelligence Operations: Potential Impact on the US Economy," Statement before the Joint Economic Committee, US Congress (May 20, 1998). Available online at: <http://www.house.gov/jec/hearings/intell/alibek.htm>.

whether conventional anthrax vaccine is effective against all the Soviet variants. However, Alibek has testified before the US Congress:

It is important to note that, in the Soviet's view, the best biological agents were those for which there was no prevention and no cure. For those agents for which vaccines or treatment existed—such as plague, which can be treated with antibiotics—antibiotic-resistant or immunosuppressive variants were to be developed.¹⁴

Moreover, there is corroborative evidence that at least some level of engineering of anthrax weapons had been attempted under the former Soviet Union. An outbreak of human anthrax in Sverdlosk (now Yekaterinberg, Russia) in April 1979 has been attributed to the accidental release of spores from a secret military microbiological facility, after a shift-worker removed a crucial filter there for several hours. Credible estimates placed the death toll in the range of 50 to 100 people, but patient hospital records were removed by the authorities. The incident was dismissed by Soviet authorities at the time as being due to the consumption of anthrax-contaminated meat from a local plant. In 1998, a study of DNA sequences extracted and amplified from preserved samples taken from eleven of the Sverdlosk victims revealed the simultaneous presence of up to four distinct genetic variants of *B. anthracis*, a finding that seems inconsistent with any kind of natural outbreak (the latter would be expected to correspond to just a single genetic variant).¹⁵ However, this interpretation has been challenged by Soviet experts. In any case, active spores were not recovered, and it is not known whether the peculiar admixture of strains that infected some of the Sverdlosk victims would

14. Ibid.

15. P. J. Jackson, M. E. Hugh-Jones, D. Adair, G. Green, K. K. Hill, C. R. Kuske, L. M. Grinberg, F. A. Abramova, and P. Keim, "PCR Analysis of tissue samples from the 1979 Sverdlosk anthrax victims: The presence of multiple *Bacillus anthracis* strains in different victims," 95 *Proc. Natl. Acad. Sci. USA* (1998), pp. 1224–9.

produce either a more virulent or more resistant form of the disease than a single “natural” strain.

The JASON List

Against this backdrop, the 1997 JASON summer study sought to identify avenues of future development for biological warfare agents. This exercise had several purposes: First, it provided an opportunity to assess the strengths and weaknesses of bioweaponry currently thought to exist.¹⁶ Second, it provided a useful framework for projecting what might someday come into existence, both through traditional approaches and through recent advances in biotechnology. Third, it helped us to consider what countermeasures might usefully be brought to bear to defend populations—or at least to minimize the damage.

In the end, we arrived somewhat arbitrarily at six broad classes of unconventional pathogens that might, or might not, come to pose a threat during the twenty-first century. This list was never meant to be all-inclusive, but only to convey a sense of the spectrum of possibilities. These cover the full range, from trivial modifications of existing pathogens to full-up synthetic diseases and life forms. At one extreme, some of the more exotic constructs may appear to be rather fanciful, and at a minimum might take considerable develop-

16. The US abandoned its offensive biological warfare program in 1969 under President Nixon, while the former Soviet Union continued biological warfare work at least through the early 1990s. Offensive biological weapons programs reportedly exist today in perhaps a dozen countries worldwide, particularly in the Middle East and Asia. Countries currently listed as “proliferation concerns” by the Henry L. Stimson Center (Washington, DC) include China, Egypt, Iran, Iraq, Israel, Libya, North Korea, Syria, and Taiwan. Today, most developed nations maintain some form of defensive-only biological warfare capability, generally integrated with their public health systems, or with military medical services.

ment effort.¹⁷ At the other extreme, some of the more straightforward modifications appear to be so obvious that they may even be under development somewhere at this moment. All would pose formidable challenges for biodefense if ever successfully weaponized and released. Finally, having gone through this exercise in imagination, I can only say that it hammers home the realization of just how redoubtable conventional pathogens are, even before they are adapted to other purposes.

Binary BW

One of the impediments to the deployment of BW is the danger they pose to those who would handle or deploy them. A binary biological weapon, by analogy with a binary chemical weapon, addresses this problem. It is fundamentally a two-component system (see figure 2-4); neither of its major parts is toxic on its own, and either one is therefore safe to handle. However, when suitably combined just prior to use, they generate a lethal mixture. How might a binary biological weapon actually work?

As it turns out, nature has already done most of the hard work of separating and compartmentalizing the needed components. Two well-characterized instances of this phenomenon are worth review-

17. In some cases, certain next-generation possibilities don't fulfill traditional criteria for being a "useful" biological warfare agent, but this fact can be misleading. *A propos* of this issue, there are plenty of existing cases of unorthodox weapons development. For example, in addition to formulating anthrax weapons, Iraq under Saddam Hussein had reportedly been developing bioweapons based on *Clostridium perfringens*, and also on aflatoxin (Source: US State Department White Paper, "Iraq Weapons of Mass Destruction Programs" [Feb. 13, 1998], available online at http://www.state.gov/www/regions/nea/iraq_white_paper.html). *C. perfringens* causes gas gangrene and presumably would tend to infect only individuals already wounded by traditional means. As for aflatoxin, a potent carcinogen, its effects can take years to develop. The tactical effectiveness of weapons based on this seemingly bizarre choice of agent has therefore been questioned. (No doubt, Saddam had something rather different in mind.)

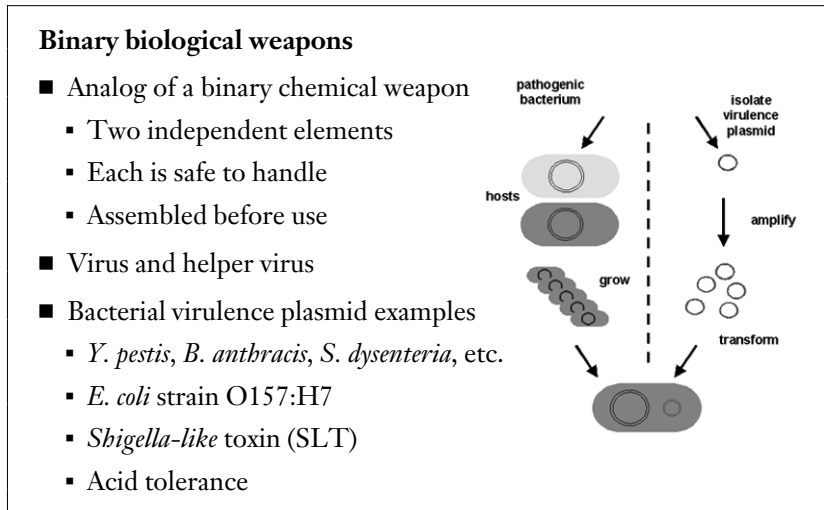


Figure 2-4

ing. The first is the case of the “satellite virus,” which is an animal virus that cannot replicate inside a host cell on its own. Instead, it needs to coinfect the host together with another virus, which codes for key proteins that the helper requires for its own propagation. The classic example is hepatitis D (also called hepatitis delta), which has the smallest known virion of any animal virus, consisting of a small circle of just ~1,636 base pairs.¹⁸ Hepatitis D needs to infect cells simultaneously with the unrelated virus hepatitis B; both are primarily transmitted through sexual contact or by contaminated blood or needles. The D virus takes advantage of the proteins expressed by the larger B virus, and greatly increases the severity of

18. Hepatitis delta is a remarkably compact RNA-based virus with special features. First, its highly abbreviated RNA message gets edited inside the cell, so that it winds up coding for two different proteins. Second, another portion of its RNA folds up to act as a *ribozyme*, or RNA-based catalyst, which is used to help with its replication. Then, like a sheep in wolf’s clothing, it covers its replicated genetic material with a coat made from hepatitis B proteins.

the disease caused by hepatitis B. Infection by hepatitis D alone is not possible.

A second and more illustrative example, for our purposes, is the virulence plasmid. Most pathogenic bacteria carry small circular DNA elements that coexist with their main chromosomes. These are called “plasmids” (or episomes). Plasmids carry an autonomous origin of DNA replication and therefore can copy themselves independently of the chromosome itself. Bacterial cells sometimes contain multiple plasmids, each coding for its own particular cassette of genes. Plasmid gene cassettes endow bacteria with specialized functions: these are not often required for bacteria to grow and reproduce in the laboratory, but they aid survival in the wild. For example, genes used by *E. coli* bacteria to conjugate (i.e., for bacterial sex) are coded by a plasmid called the F' element. As it happens, the genes that cause virulence in bacteria are most often found on plasmids; this happens to be the case for the plague (*Yersinia pestis*), anthrax (*Bacillus anthracis*), dysentery (*Shigella dysenteriae*), and other diseases. Both in the wild (by mechanisms unknown) and in the laboratory (through simple biotechnology), plasmids can be transferred among different kinds of bacteria across species barriers. Bacterial cells can also be “cured” of their plasmids, i.e., caused to lose them altogether.

One infamous example of a virulence happens in *E. coli* bacteria. Everyday strains of *E. coli*—such as the ones we have in our gut—don't produce toxins, but at least half of the known strains do. These toxins include various colicins, special molecules that target competing bacteria. Certain *E. coli* toxins cause forms of gastroenteritis in humans, but most of these are not life-threatening. However, there is a notable exception. In the case of *E. coli* strain O157:H7, the organism somehow picked up a rogue plasmid coding for a *Shigella*-like toxin (SLT) that produces a potentially deadly form of *hemorrhagic* enteritis when these bacteria multiply in the intestines. It is not yet known exactly where this plasmid came from, nor how it came to reside in this formerly benign strain. Not only does *E. coli*

O157:H7 code for a lethal toxin, but it has also developed the unusual ability to live in rather acid environments (down to pH 5.0), such as those found in apple cider or cured sausage. These food products normally have long shelf-lives and do not require sterilization. The result is that a commonplace bacterium emerged as a killer disease—and unpasteurized cider has become a thing of the past.

Taking our cue from nature, a binary biological weapon could be produced roughly as follows. First, a virulence plasmid is isolated from a pathogenic parent strain and an antibiotic resistance gene is introduced into it using standard molecular biology techniques. The plasmid is then amplified directly, using biosynthetic-based methods,¹⁹ such as the polymerase chain reaction (PCR). Independently, the original parent strain is cured of its plasmid, and the resulting nonvirulent isolate is cloned and grown up. Both components are individually harmless, and can therefore be handled in significant quantities without risk. The final step comes just before the weapon is deployed, and consists of transforming the host strain back into a pathogen. In practice, there are many ways to accomplish such a transformation. Perhaps the simplest is to treat the bacteria briefly with a solution of calcium chloride, which makes their cell walls leaky. With small probability (roughly 1 in 100,000), the plasmid DNA is taken up by the cell. If treated cells are then grown up in a medium containing the right antibiotic, only those organisms that have been successfully transformed will propagate, since the virulence plasmid also confers antibiotic resistance.

In actual practice, the final transformation (i.e., the combination of cells and plasmids) and subsequent regrowth phase would pre-

19. Alternatively, the plasmid could be reintroduced into a different host where it is rendered nontoxic but can nevertheless replicate successfully. The host is then grown up in large quantities, broken apart, and its plasmid DNA isolated. The choice of method depends, in part, on the size of the plasmid(s) involved.

sumably take place inside a small bioreactor that constituted the weapon itself, perhaps the size of a beer can. Because of their non-toxicity, all bioreactor ingredients may be handled with safety. The deployer of the weapon need not even be present to initiate the final transformation/regrowth phase, which would be accomplished automatically upon triggering the device.

There are many variations on this basic theme, including the use of multiple plasmids to reconstitute the pathogenic strain (the plasmid may be so small as to carry only a single critical gene, for example), or the use of a host strain that is genetically distinct from the parent originally used to isolate the virulence plasmid. The key point is that a binary weapon makes it feasible to grow up kilogram quantities of reagents without posing an undue risk to its manufacturer.

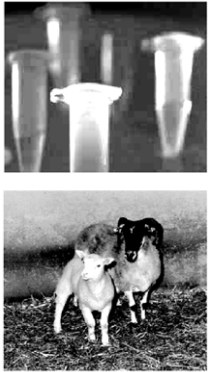
Designer Genes and Life Forms

The success of genome projects worldwide has led to knowledge of the complete genetic codes for literally hundreds of viruses, including most of the significant viral pathogens infecting humans. Today, we also know the DNA sequences of several dozen microorganisms, including *E. coli* and yeast. These microorganisms include many of the major bacterial pathogens (and potential biowarfare agents) such as *Yersinia*, *Salmonella*, *Streptococcus*, *Listeria*, *Leishmania*, *Legionella*, *Clostridium*, and others.²⁰ Recently, the first sequence of an entire multicellular organism was completed, the nematode worm *C. elegans*.²¹ In a short time, the sequences for the fruit fly (*D. melanogaster*), mouse, and the human will be finished.

20. An updated listing of known bacterial sequences is maintained by the Institute for Genome Research (TIGR, Inc.) and available online at <http://www.tigr.org/tdb/mdb/mdb.html>

21. December 11, 1998 special issue; multiple articles. 282 *Science*, pp. 2011–46.

Designer genes and life forms



- Synthetic genes and gene networks
 - Combinatorial strategies: DNA shuffling (“sexual PCR”)
 - 32,000 × β -lactamase
- Synthetic viruses
 - Influenza antigen switching, N3/H6
- Synthetic organisms
 - Chimeras and de novo design
 - Mycoplasma genitalium* (541 genes, ~0.6 MB)
 - Mycoplasma pneumoniae* (677 genes)
 - Estimate ~250–400 genes needed

Figure 2-5

All this new information raises the very real possibility of developing (in increasing complexity) synthetic genes, synthetic viruses, and even entire synthetic organisms. All three possibilities represent potential points of departure for would-be biological warfare developers (see figure 2-5). Perhaps the most straightforward—and arguably the most effective—way to increase the effectiveness of any bacterial warfare agent is to simply render it resistant to known antibiotics. A number of antibiotic-resistance genes already exist in nature: these include a gene that codes for the protein β -lactamase, which can break down penicillin. But the administration of large doses of antibiotic (or structural variants of that antibiotic) can overwhelm the activity of many natural resistance genes, and can therefore serve as an effective treatment. Using newly developed combinatorial strategies, however, it has become possible to carry out “evolution” in a test tube, and generate supergenes coding for antibiotic-destroying proteins with preternatural activity. The technique of DNA shuffling (also called “sexual PCR”) developed by W.

P. Stemmer and colleagues is one such strategy.^{22,23} The method is based on random recombination and reassembly of existing fragments of genes amplified by PCR. With appropriate selection, it is possible to evolve in just a few iterations a modified β -lactamase that works $\sim 32,000$ -fold faster than the natural version, and is therefore capable of coping with correspondingly higher levels of antibiotics. This finding has serious implications for biological warfare: Organisms equipped with a whole bank of antibiotic-resistant supergenes might be impossible to stop. But DNA shuffling—like so many other powerful biotechniques—is a two-edged sword. It also holds the promise of developing organisms with genes that could detoxify poisonous wastes, such as arsenic²⁴ or oil spills. It might equally well be used to develop improved, recombinant vaccines that could play a major role in defense against biological warfare.

Synthetic viruses have now become possible, and once again nature points the way. Consider influenza, which despite its nuisance reputation in the workplace can be a deadly killer, as the pandemic of 1918 amply demonstrated, producing more deaths than all the battles of World War I. The flu is an extremely mutable and virulent virus. The variability of influenza is the reason for annual flu shots. The virus is able to mutate sufficiently rapidly during the course of a year that antibodies raised against the previous year's strain become largely ineffective. Influenza does this, in part, by periodically swapping out entire genes and replacing them with var-

22. W. P. Stemmer, "Rapid Evolution of a Protein in Vitro by DNA Shuffling," 370 *Nature* (1994), pp. 389–91. See also <http://www.maxygen.com>.

23. A. Cramer, S. A. Raillard, E. Bermudez, and W. P. Stemmer, "DNA Shuffling of a Family of Genes from Diverse Species Accelerates Directed Evolution," 391 *Nature* (1998), pp. 288–91.

24. A. Cramer, G. Dawes, E. Rodriguez, S. Silver, and W. P. Stemmer, "Molecular Evolution of an Arsenate Detoxification Pathway by DNA Shuffling," 15 *Nature Biotechnology* (1997), pp. 436–8.

iant forms. For the flu, the two main surface antigens are neuraminidase (N) and hemagglutinin (H). Gene swapping is thought to come about in a natural way. There are many forms of the flu in animals, including an avian form affecting birds such as geese and ducks, a swine form affecting pigs, and a human form affecting ourselves. Pigs can harbor *both* the avian and human forms, in addition to their own. In situations where ducks, pigs, and humans live in close proximity and under poor sanitary conditions (for example, on small farms in China), a pig can sometimes become infected simultaneously with two or more influenza strains, including one from ducks and one from humans. With some probability, the genes occasionally get mixed up inside pig cells and the result is the emergence of a novel, recombinant virus with a subset of components derived from each strain.

A bioweapons developer inspired by this state of affairs might attempt the *ab initio* construction of a synthetic virus using a kind of “erector set” strategy, building it up by literally mixing and matching known components of existing viruses. Alternatively, it may be sufficient to subtly alter the surface properties of the major antigens (the H and N proteins in flu, for example, for which many variants are known) in such a way that the virus retains virulence but loses an ability to be recognized by the immune system. In either case, the result could be a new human disease. Some animal viruses are so small that their entire genome can be stitched together, at least in principle, from machine-synthesized fragments using current technology. This raises the intriguing possibility that a virus could be created entirely from scratch, so to speak, rather than made by a cell.

The smallest known organisms are the mycoplasmas, tiny gram positive-like bacteria whose genomes are a scant ~0.6 megabase pairs (compare with *E. coli*, which is almost eight times larger, at 4.6 megabase pairs). The first mycoplasma to be sequenced, *Mycoplasma*

genitalium, has just 541 genes.²⁵ The second mycoplasma, *Mycoplasma pneumoniae*, carried 677 genes,²⁶ and homologues of every one of the 541 genes from its relative were contained within that number (the rest are presumably nonessential). This fact has led to the speculation that as few as 250 to 400 genes may be required to create a complete organism. If the actual number turns out to be that small, we may not be so far from the day when creating an entirely synthetic organism becomes feasible. This, of course, has obvious implications for biological warfare.

Gene Therapy as a Weapon

The goal of gene therapy is to effect a change in the genetic makeup of an individual by introducing new information designed to replace or repair a faulty gene (see figure 2-6). In principle, gene therapy could be used to treat an enormous number of human diseases and conditions known to be associated with specific genetic defects, including diabetes, heart disease, cystic fibrosis, muscular dystrophy, immune disorders, hereditary anemias, cancer, and even mental illness. In view of its nearly unlimited and untapped potential to alleviate human suffering, it represents the Holy Grail of modern medicine. Small wonder that so many clinical and biotechnical programs now focus on gene therapy research. Gene therapy promises unimaginable benefits for mankind!

Broadly speaking, there are two classes of gene therapy: germ-line and somatic cell. Replacing or augmenting the DNA of a germ-line cell would, in principle, lead to a heritable change that could repair problems for all future generations. Somatic cell therapy, in

25. C. M. Fraser, et al., "The Minimal Gene Complement of *Mycoplasma genitalium*." 270 *Science* (1995), pp. 397-403.

26. R. Himmelreich, H. Hilbert, H. Plagens, E. Pirkl, B. C. Li, and R. Herrmann, "Complete Sequence Analysis of the Genome of the Bacterium *Mycoplasma pneumoniae*." 24 *Nucleic Acids Res.* (1996), pp. 4420-49.

Gene therapy as a weapon

- Goal: effect a permanent change in genetic makeup
- Approach: use transforming viruses or similar DNA vectors carrying “Trojan horse” genes: e.g., retrovirus, adenovirus, poxvirus, HSV-1, etc.
- Potential for misuse
 - Intentional
 - Unintentional



Figure 2-6

contrast, affects only the cells of the individual receiving it. For technical as well as ethical reasons, most current research has concentrated on the development of somatic cell gene therapy.

Gene therapy has additional prospects: It may be used as a kind of drug delivery system, to supply cytokines, blood clotting compounds, hormones, or other factors. It may equally well be used as a form of “vaccine,” to confer immunity to certain diseases, such as AIDS. Finally, it could be used to delete (knock out) genes as well as to introduce them, or to change the expression patterns (or levels) of any genes.

Unfortunately, although there have been isolated and largely anecdotal successes, no one has any kind of reliable gene therapy working just yet. But it wouldn't be unreasonable to assume that some forms of gene therapy will become available early in the next century. Although complicated in practice, the idea behind gene therapy is simple: First you have to get the gene of choice into cells, and then you have to arrange for the gene to be stably expressed at

the right levels. (This second bit is where most attempts fail, incidentally.) There are quite a few ways to go about doing this, in principle—including just adding “naked” DNA to cells and waiting for them to take it up. More often, though, a crippled virus is modified to serve as a kind of Trojan horse, or genetic vector. The virus is designed to retain its capacity to attach to and infect cells, and in certain cases to replicate or perhaps even to spread from cell to cell. But, importantly, the vector lacks critical genes that lead to viral disease. The gene of choice is then introduced into the virus vector along with special gene control elements designed to function once the vector reaches its cellular target. A great number of vectors are presently being constructed and tried. Some are based on transforming viruses, such as adenovirus (which causes disease in monkeys) and defective pox or herpes viruses. These animal viruses can exist for long periods inside our cells, but their DNA usually remains separate from the cell’s own chromosomes. This separate identity can lead to eventual gene loss or instability. For this reason, some labs have chosen to work with an adeno-associated virus (AAV), which can integrate itself into chromosomes with some probability, to establish a latent state. Other labs are working to manufacture vectors based on retroviruses (such as the HIV virus that causes AIDS), which can permanently integrate single copies of themselves into the chromosomes, with a view to affording a more permanent and regulated transformation. It would be a singular irony if the great remedies of the twenty-first century were based on the last untreatable disease of the twentieth century.

Regardless of the vector technology that eventually will be developed, it seems clear that successful gene therapy is yet another example of a two-edged sword, with equal potential for misuse. Genes can be introduced for good or for evil, and they can induce disease as well as cure it. It has been speculated that some gene therapy vectors may be so easy to introduce that they will come in the form of a simple nasal spray. One quick whiff in a nostril trans-

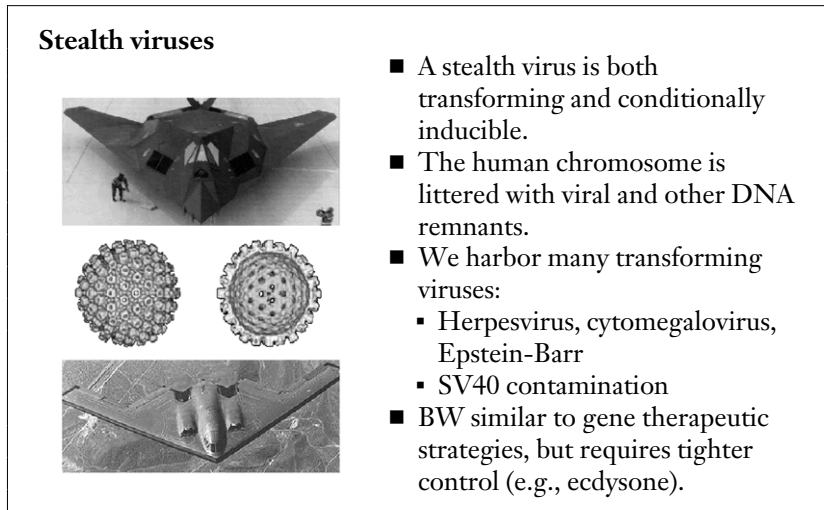


Figure 2-7

fers the virus in aerosol form, and the vector takes care of the rest. If this sort of thing comes to pass, gene therapy vectors might be subverted to become potent BW of destruction.

Stealth Viruses

A “stealth virus” is another menacing possibility afforded by genetic engineering (see figure 2-7). The basic idea behind a stealth virus is to produce a tightly regulated, cryptic viral infection, using a vector that can enter and spread in human cells, remaining resident for lengthy periods without causing detectable harm. However, once triggered by an appropriate external (or internal) signal, the cryptic virus is activated and causes disease. Stealth viruses could be designed to be contagious, and therefore distribute themselves silently throughout a given population. They might even be designed against specific target groups. A population could be slowly preinfected with a stealth virus over an extended period, possibly years in

advance, and then synchronously triggered. Stealth viruses therefore have utility beyond that of traditional bioweapons. For example, they could be disseminated and used to blackmail a population based merely on the threat of their activation.

At its core, a stealth virus is not so very different from any of the gene therapy vectors discussed in the previous section. It is based on the realization that most humans already carry a substantial and silent viral load.²⁷ For example, a significant fraction of the human population already carries herpes simplex (which in one of its forms causes cold sores), Epstein-Barr virus (which causes mononucleosis, or glandular fever), or human cytomegalovirus (found in up to 90 percent of urban populations). Some resident viruses, for example *herpes simplex I*, infect permanently but only produce symptoms periodically. Most of the time, the virus lies dormant inside cells (often in the trigeminal facial nerve, in the case of oral herpes), waiting to be triggered by some kind environmental assault. Examples of such assaults include cuts, chafing, exposure to ultraviolet rays (sunburn), infection by an unrelated disease, and physical or mental stress. The mechanism for triggering an active herpes infection is currently a subject of study.

Another example of a cryptic human infection, which remains a topic of controversy, dates to the widespread administration of Sabin type polio vaccines between 1955 and 1961.²⁸ These vaccines were prepared using live African green monkey kidney cells, and batches of polio vaccine became contaminated by low levels of a

27. In addition to silent (and complete) viruses, it has been estimated that the human genome is littered with various remnants of viruses from our past, amounting to 3 percent or more of the total DNA. This is probably true of all organisms, in fact. When the DNA sequence of *E. coli* was determined, it was found to harbor dozens of cryptic prophages, inactive remnants of bacterial viruses. F. R. Blattner, et al., "The Complete Genome Sequence of *Escherichia coli* K-12," 277 *Science* (1997), pp. 1453–62.

28. K. Shah and N. Nathanson, "Human Exposure to SV40: Review and Comment," 103 *Amer. J. Epidemiol.* (1976), pp. 1–12.

monkey virus, simian virus 40 (SV40), which eluded the quality control procedures of the day. As a result, large numbers of people—probably millions, in fact—were inadvertently exposed to SV40. The virus has since been shown to produce cancer in hamsters, although it does not do so in monkeys. It can also recombine with other monkey viruses, such as adenovirus. Evidently, however, the virus did not produce widespread problems for humans, and so my own generation may have dodged a bullet. Nevertheless, there is evidence that SV40 virus still exists in certain individuals exposed to the contaminated polio vaccine, and a great deal of speculation occurs about whether it may be responsible for some disease.

Taking a cue from this history, a stealth vector design could be based on one or more existing examples of dormant virus. In key respects, a stealth virus resembles a gene therapeutic vector. Just as in gene therapy, the vector must be able to enter the host and effect a stable transformation of cells. And just as in gene therapy, certain critical genes of the vector (in this case, those that lead to disease) must be regulated, so that their expression levels can be controlled. In contrast with gene therapeutic vectors, however, the genetic control must be extraordinarily tight, so that a potentially lethal disease is not released prematurely, but only in response to the desired trigger. This kind of exquisite control has not been easily achieved with genetically engineered systems, which tend to be “leaky.” Recently, all that has changed with the advent of new, tighter control systems, such as those based on the insect hormone ecdysone.²⁹

Host-Swapping Diseases

Viruses are basically parasites. They are symbiotic, and coevolve with their living hosts, depending upon them to furnish most of

29. D. No, T. P. You, and R. M. Evans, “Ecdysone-inducible gene expression in mammalian cells and transgenic mice,” 93 *Proc. Natl. Acad. Sci. USA* (1996), pp. 3346–51.

Host-swapping diseases

- Parasites develop in evolutionary “equilibrium” with their hosts.
- Pathogens have narrow host ranges:
 - Equine encephalitis
 - Influenza, smallpox
 - CJD, BSE
- Example: *Canine parvovirus*
 - *Feline panleukopenia virus* + 2 capsid protein mutations (appeared ~1974)
- Disruption of equilibrium tends to produce nothing, *or* high virulence!
 - AIDS
 - Hantavirus
 - Marburg, Ebola

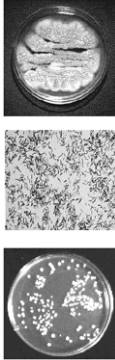


Figure 2-8

what is required to grow and propagate. Any virus that produces a disease so deadly that it kills off its host is committing suicide, because it rapidly comes to an evolutionary dead end. Indeed, any virus that even *distresses* its host to any significant degree will cause that same host to lose critical natural fitness, leading to another evolutionary dead end—albeit a slower one. Why, then, do viruses even cause disease? Perhaps surprisingly to some, the vast majority of viruses do not cause disease; they are utterly silent, in the sense discussed earlier. It is only the rare virus that disrupts the fragile evolutionary “equilibrium” between parasite and host. This can happen, in particular, when a virus skips out of its host range and accidentally targets a different species (see figure 2-8). It can also happen when a new virus is inadvertently created from the components of other viruses, or mutates, or picks up some genes by mistake. Viral diseases typically have short lifetimes on an evolutionary time scale. Smallpox, for example, is thought to have emerged in the modern human population only between three and ten thousand years ago.

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Animal viruses tend to have narrow, well-defined host ranges—often just one to a few species. One example discussed earlier is human influenza. Another is SV40, which causes disease in hamsters but not in humans. Animal viruses tend to have a natural animal reservoir where they reside and cause little or no damage. The virus responsible for Eastern equine encephalitis (EEE), for example, grows mainly in water fowl, but it can be transmitted by mosquitoes that bite birds and then transfer it to horses or humans, where it can cause a fatal (but not contagious) disease. The animal reservoir for the deadly (to humans) hantavirus is the rodent, especially deer mice. The animal reservoir for Ebola virus is currently thought to be the bat,³⁰ where it can grow to high blood levels (titers) but does not apparently produce symptoms. Quite recently, it was established that the original source of the AIDS virus is likely to be the chimpanzee.³¹ However, that virus has now mutated in humans to a form that causes AIDS even in chimps.³²

The lesson, so to speak, is that when viruses are transferred out of their natural host reservoir, they tend to do one of two things. With high probability, they remain inactive and unable to propagate in a different host species. With lower probability, they can gain a toehold. Frequently, in such situations, they cause disease. Viruses can also jump out of their host range by acquiring one or more mutations that allow them access to cell surface receptors of a new host. An example of this is canine parvovirus, against which most pet dogs are now routinely vaccinated by veterinarians. Parvovirus is a deadly disease of dogs, but it was utterly unknown before about 1974. Around that time, a feline panleukopenia virus (cat distemper)

30. R. Swanepoel, et al., "Experimental Inoculation of Plants and Animals with Ebola Virus," 2 *Emerging Infectious Diseases* (no. 4) (1996), pp. 321–5.

31. F. Gao, et al., "Origin of HIV-1 in the chimpanzee *Pan troglodytes*," 397 *Nature* (1999), pp. 436–41.

32. F. J. Novembre, et al., "Development of AIDS in a Chimpanzee Infected with Human Immunodeficiency Virus Type 1," 71 *J. Virol.* (1997), pp. 4086–91.

somehow acquired just two capsid protein mutations, thereby turning it into a lethal dog disease. Since then, parvovirus has continued to evolve slightly, picking up a couple of additional mutations. There are many kinds of parvovirus in nature, including several variants that infect humans.


A determined developer seeking to create a new biological weapon might take inspiration from all this. If the proper mutations were introduced into a relatively benign animal virus, permitting it to escape from its normal host range and infect humans instead, it would have the potential to become a virulent human disease. Several highly lethal viral diseases, such as AIDS, Ebola and Marburg fevers, and hantavirus are now classified by the CDC as *emerging diseases*: They all qualify (more or less) as examples of this very phenomenon—only the “experiment” was performed by nature in this case, and not by the hand of man! However, with stringent genetic selection and appropriate biotechnology, it does not seem so far-fetched to suggest that the same type of experiment might be accomplished in the laboratory. The result, of course, would be a man-made emergent disease.

Designer Diseases

Our understanding of cellular and molecular biology is nearly to the point where it may be possible to contemplate a disease first, then construct the pathogen necessary to produce it second (for example, using one of the vectors discussed earlier). Such a “designer disease” might work through any of a variety of molecular signaling pathways that are critical to the health of humans. Like the HIV virus that causes AIDS, a disease might target the immune response, escaping our natural ability to fight it; or it might activate normally dormant genes and wreak havoc in our cells; or it might simply instruct the cells in our body to commit suicide, as follows.

Programmed cell death, or “apoptosis,” is a facet of nature (see

Designer diseases: Subversion of signal transduction pathways



- Apoptosis: Programmed cell death
 - Organismic development (e.g., limb growth, *C. elegans* lineage)
 - Tissue identity (e.g., NGF requirement)
 - Tumor suppression (e.g., p53)
- Specific apoptosis surface receptors: “death receptors”
 - e.g., Fas, TRAMP, TRAIL-R, TNF-R1
 - Most ligands not yet identified
 - Cytokine receptors
- General agonist would be lethal
- More “nuclear weapons”: other signaling pathways
 - Immune system, growth regulation, etc.

Figure 2-9

figure 2-9). Human cells, it would seem, all contain one or more built-in signaling pathways which, when suitably activated, cause them to die. Apoptosis serves many useful purposes in organisms, from development to fighting cancer. For example, embryonically, human babies start out with webbed hands and feet. At a later stage, cells in the webbed areas are programmed to die off, leaving us with five distinct digits on each appendage. This kind of cellular die-off is critical to our forming human shapes, of course.

Apoptosis is also used in an entirely different way. When cells develop certain types of chromosomal instability or mutation, as is frequently the case in cancer or during viral infection, a *tumor suppressor* pathway is activated that causes the cells to chop up their own DNA into small bits and commit a very characteristic form of suicide. This pathway is mediated by a set of supervisory genes called *tumor suppressor genes*, the best known example of which is p53. Normally, once cancer begins to take over a cell, a tumor suppressor like p53 directs it to die altruistically—halting the transformation. But a

loss of p53 function means that organisms lose their innate ability to stop the spread of cancer. That is why the p53 gene is found to be missing or mutated in so many forms of human cancer (up to 65 percent for colorectal cancer, 70 percent for lung cancer, and 60 percent for skin cancer).³³

Tumor suppressors represent one set of genes involved in apoptosis, but there are others. Nerve cells grown in culture, for example, commit apoptosis unless a special small molecule, nerve growth factor (NGF), is supplied to the medium. Put differently, nerve cells are prepared to die the very moment they are deprived of NGF. They sense the presence of NGF levels via specific receptors in their membranes that bind to external NGF and signal to the rest of the apoptotic pathway. In other types of cells, death can be induced by the *addition*, rather than the deprivation, of other small signaling molecules, such as cytokines or necrosis factors.

Because of its intimate relation to cancer, apoptosis has been the subject of an enormous amount of recent work, and quite a few cell receptors have already been identified, with strange names (which keep changing) like Fas, TRAMP, TRAIL-R, and TNF-R1. These all hook up to a variety of “death pathways” that use a common mechanism to kill the cell.³⁴ Many of their natural ligands remain to be identified, however.

A designer disease might be created, for example, by subverting one or more of these apoptosis pathways. If a *general agonist* for apoptosis receptors were identified, i.e., a small compound that activated death pathways in all cells, it would potentially be more toxic than any known poison! A biological warfare viral vector designed

33. A database of human mutations is maintained at: <http://www.umd.necker.fr/disease.html>. A p53 database with frequency information can be found at: http://perso.curie.fr/Thierry.Soussi/p53_database.html.

34. C. M. Rudin and C. B. Thompson, “Apoptosis and Disease: Regulation and Clinical Relevance of Programmed Cell Death,” 48 *Annual Rev. Med.* (1997), pp. 267–81.

to direct the synthesis of such an agonist would lead to the mass suicide of otherwise healthy cells—and ultimately to the death of the organism.³⁵ This is only one kind of designer disease: quite a few others can be conjured up based on similar principles.

Concluding Remarks

This, then, is the *real* Y2K problem, or “millenium bug!” How do we protect ourselves from ourselves as we enter the next millennium? Like the nuclear threat, the specter of biological warfare presents a multifactorial problem, and it will call for multifactorial solutions. No one single remedy seems likely to make this thing go away. And, like the nuclear genie released from the bottle, the newfound power unleashed by advances in biotechnology will not be tamed by merely wishing it—or legislating it—away. Once knowledge is attained, there is no going back.

A large number of government and government-affiliated agencies are now investigating aspects of the biological warfare threat and developing specific recommendations. The JASON group, for example, developed its own set during the 1997 Summer Study. Rather than repeat these here, I will conclude by touching briefly on just some of the challenges faced in coping with the next generation of pathogens (see figure 2-10).

Anticipation and Detection

Identification and classification. We urgently require newer, faster, and more reliable ways to identify viruses and bacteria—both traditional and next-generation pathogens. We must also develop im-

35. Of course, no such general agonist may actually exist, for any of a variety of excellent reasons. Even so, the widespread—and therefore inappropriate—expression of the specific ligand for *any* of the many apoptosis pathways may be more than sufficient to cause disease.

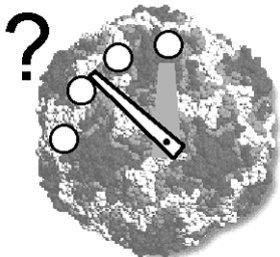
<p>The true Y2K problem!</p> <ul style="list-style-type: none"> ■ Anticipation and detection <ul style="list-style-type: none"> ▪ Identification and classification: both old and new threats ▪ Screening: develop and test indicators of threats ▪ Vigilance: use of indicators to detect threats ■ Mitigation and remediation <ul style="list-style-type: none"> ▪ New vaccines, antibacterials, antivirals, anti-? ▪ Medical infrastructure and response ■ Political and military response <ul style="list-style-type: none"> ▪ The nuclear threat redux—but worse! ■ Education: <i>A Call from Arms</i> <ul style="list-style-type: none"> ▪ Current lack of awareness and responsibility in the professional community ▪ Bioethics training beyond Dolly 	 <p>The Doomsday Clock: Ticking since June 1947. Reset on June 11, 1998, to midnight minus 9 minutes.</p>
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Figure 2-10

proved ways of collecting, saving, and preparing biological samples. One sensitive method that holds great promise is based on PCR amplification of target DNA (or RNA, using RT-PCR). We need to develop ways to miniaturize and automate bio-detectors, and make these ready for use in the field, as well as in the laboratory. We also need to explore other promising technologies, including various forms of hybridization array, immunological detection methods, light- or mass-spectrometry, and so forth. Perhaps most important of all, we need to be able to anticipate the detailed nature and scope of next-generation biological warfare threats. Anticipation holds the key to detection: One can't readily develop a test for something one's not expecting to find! We need to try to stay one or more steps ahead of an adversary.

Screening. We need to be thorough in screening for presumptive pathogens throughout the whole of the environment. This implies not only developing and deploying appropriate sensors and detectors, but also becoming systematic about testing these and un-

derstanding their intrinsic limits. Moreover, “detectors” need not be limited to technology alone. There is a real and growing need for human intelligence as it relates to biological warfare, and this seems likely to remain a prime source of information.

Vigilance. Having produced detectors, we need to use these consistently and wisely. Perhaps more importantly, we need to update regularly our detectors,³⁶ to take advantage of the latest intelligence and technical developments. This is nontrivial.

Mitigation and Remediation

We must develop new vaccines, new antibacterials, and new antivirals. We must also develop entirely new ways to make such things. We must improve upon existing remedies. Much of this work will fall to the pharmaceutical and biotechnology sectors, which are already active in this area. The one saving grace of this whole gloomy scenario may be that the very same technologies that make possible BW may make it possible to defeat them.

The public health response to BW threats needs to be addressed and coordinated at federal, state, and local levels *as never before*. Biomedical, military, and legal authorities will all need to become involved. We need to produce and stockpile whatever materials will be needed for rapid deployment of health resources. We may also need to stockpile certain vaccines, or to develop a rapid pipeline for manufacture and delivery of them. We may even consider large-scale vaccination programs, but this remains to be seen. Finally, we need to give careful thought to implementing health measures that are consistent with our traditional constitutional protections and personal freedoms.

36. For the case of PCR-based biosensors, these devices are no better than the *primers* supplied (primers are short DNA fragments specific to the target, used to initiate the amplification of sequences). Particular care must be given to the design and selection of primers, and this will require an ongoing effort in the face of a changing biological landscape.

Political and Military Response

In many respects, the BW threat is just like the nuclear threat—*only worse!* It poses significantly greater risks for the proliferation and control of arms. Our nation will need continually to revise its guidelines for political and military responses to BW use. The US should take a leadership position in helping to strengthen the provisions of the international Biological and Toxin Weapons Convention (BWC) to include meaningful, multilateral inspections. The worldwide ban on biological weaponry must be enforceable.

Education: A Call from Arms

I should like to close with a personal observation. There is a regrettable lack of involvement by members of the professional biomedical community in issues related to BW, particularly among molecular biologists working in industry and academia. Some of this is understandable, because it is an exceedingly unpleasant topic to contemplate. But I believe the issue goes much deeper than that. Some folks simply do not take the threat seriously, but they should. Others worry about provoking a widespread public backlash against biotechnology in general that might have a chilling effect on their own legitimate biological research. Still others worry that by calling “unnecessary” attention to the problem, they will paradoxically hasten its development. None of these excuses stands up to close scrutiny.

When the National Institutes of Health began work on the Human Genome Project, it set aside 5 percent of all funding to the National Human Genome Research Institute (NHGRI) for a program in the Ethical, Legal, and Social Implications of the work (ELSI, established in 1990).³⁷ Partly in response, most major univer-

37. The US Department of Energy, which also funds work on the Human Genome Project, also sets aside funding for the same purpose.

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sities receiving federal funds now offer biomedical ethics courses for graduate students and postdocs receiving training in molecular biology. These courses cover many timely and important topics, such as scientific misconduct, privacy issues related to DNA and human health, and the ethics of animal cloning. Rarely, however, do they attempt to educate young professionals on ethical issues related to BW. It is high time to offer biomedical ethics training “beyond Dolly.” Biological scientists at all levels need to get involved. To quote Preston:

The community of biologists in the United States has maintained a kind of hand-wringing silence on the ethics of creating bioweapons—a reluctance to talk about it with the public, even a disbelief that it’s happening. Biological weapons are a disgrace to biology. The time has come for top biologists to assert their leadership and speak out, to take responsibility on behalf of their profession for the existence of these weapons and the means of protecting the population against them, just as leading physicists did a generation ago when nuclear weapons came along. Moral pressure costs nothing and can help; silence is unacceptable now.³⁸

Amen! As George Orwell put it, “Life is a race between education and catastrophe.”

38. Richard Preston, “Taming the Biological Beast” Op-Ed, *New York Times* (Apr. 21, 1998).