The NIAID Research Agenda on Biodefense

The National Institute of Allergy and Infectious Diseases faces new challenges in fighting the war on bioterrorism

Tara Palmore, Greg Folkers, Carole Heilman, John R. La Montagne, and Anthony S. Fauci

A terrorist attack on the United States using biological agents was once thought to be a remote and distant possibility. Since 1990 two major events, the fall of the Soviet Union and the Gulf War, began to transform this assessment. It became known following the Gulf War that Iraq had established a very ambitious and well-financed program to develop various weapons of mass destruction, including sophisticated biological and chemical weapons. While the United States had abandoned the development of biological weapons in 1972, it was sobering to learn that the Soviet Union had not, and, in fact, continued to produce vast stores of smallpox and other potential agents of bioterrorism. The increasing number of reports revealing the magnitude of international biological weapons programs catalyzed the emergence of a political and scientific consensus on the priority of biodefense research. The destruction of the Murrah Building in Oklahoma City, the bombing of U.S. embassies in Nairobi and Dar es Salaam, the near sinking of the U.S.S. Cole, and the attacks on the World Trade Center and the Pentagon on 11 September 2001 have led to a reassessment and realignment of efforts within the United States to prepare for the likelihood of additional terrorist attacks, including those involving biological weapons. Perhaps most significantly, the anthrax attacks in the fall of 2001 transformed the priorities of the American public, government, and science community.

In particular, heightened awareness of the potential for bioterrorism prompted a remarkable response from scientists around the world. The National Institute of Allergy and Infectious Diseases (NIAID) received an unprecedented number of offers from the research community interested in providing ideas and services for the nation's biodefense efforts and proposing steps to combat the threat of biological weapons. Emerging diseases and bioterrorism research became an even greater focus of NIAID's Division of Microbiology and Infectious Diseases and Division of Intramural Research. While the study of the potential agents of bioterrorism has been on NIAID's agenda for many years, the research agenda has now been galvanized by an unprecedented sense of urgency. In February 2002, a blue ribbon panel of experts was assembled to help define the Institute's research agenda for the Centers for Disease Control and Prevention's (CDC) Category A agents (www.niaid.nih.gov). The panel members produced two documents that contain the biodefense strategic plan and the Category A research agenda (Fig. 1).

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Research Programs and Priorities

NIAID's biodefense research program balances basic and applied research, with an emphasis on

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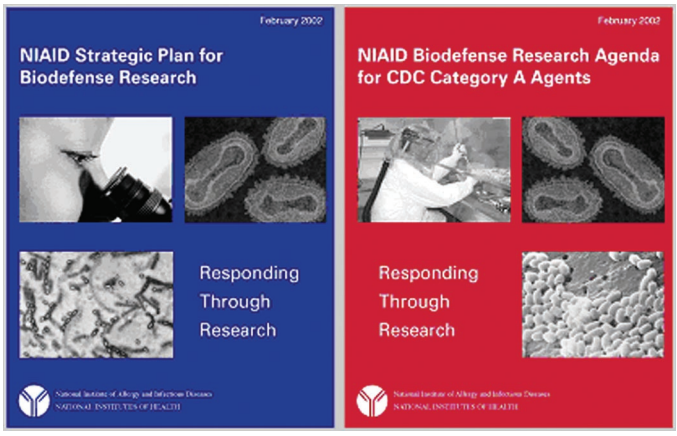
FIGURE 1



The NIAID biodefense research strategy was formulated in February 2002, with input from a blue ribbon panel of experts. The Strategic Plan (left) outlines plans for addressing research needs in the broad area of biodefense and emerging and reemerging infectious diseases. The Research Agenda (right) focuses on the goals for addressing Category A agents (smallpox, anthrax, plague, tularemia, botulism and viral hemorrhagic fevers).

the rapid development and delivery of products that could be used to combat agents of bioterrorism. The Institute's research portfolio includes—and extends beyond—the potential bioterror agents in Category A (i.e., the agents of smallpox, anthrax, plague, tularemia, botulism and viral hemorrhagic fevers). It includes investigations into a broad range of potential pathogens. The strategy for all aspects of NIAID's approach to bioterrorism preparedness involves cross-cutting research and development goals that approach each pathogen from multiple perspectives. The Institute's biodefense research agenda focuses on six interrelated areas: microbial biology, host responses to microbes, vaccines, diagnostics, therapeutics, and research resources.

● **Microbial biology.** Important research goals include attaining a better understanding of microbial virulence factors and pathogenesis, genomic sequencing of multiple strains of bacterial pathogens using new technology, and studying microbial proteomics. For example, The Institute for Genomics Research (TIGR), with NIAID support, is sequencing the genomes of several strains of *Bacillus anthracis*; these data will be useful for comparative genomics, virulence, and diagnostics research and will have obvious forensic value. In recent months, NIH-supported scientists have discovered the structure and function of the three anthrax toxins, fueling active public and private sector pursuit of antitoxin therapies. Basic research on the biology and infectivity of Category A, B, and C pathogens will provide a deeper knowledge of those pathogens, similar to what has already been learned about *B. anthracis*.

● **Host responses.** There is much progress to be made in understanding how innate and adaptive immunity affect the pathogenesis of viral and bacterial infections. In addition, the relationship and interdependence of the innate and adaptive immune responses are areas of potentially fruitful research. We know that in some diseases, such as viral hepatitis, the immune response causes more damage than the pathogen itself. In others, like anthrax, toxic bacterial products progressively degrade the ability of phagocytes to destroy the bacteria, such that systemic infection rapidly leads to overwhelming bacterial multiplication and sepsis. Research on the human host responses, long a priority, has been accelerated with the aim of developing immunomodulatory therapies and producing more effective vaccines.

● **Vaccines.** NIAID has taken steps to hasten the development and production of vaccines for prophylaxis against a broad range of pathogens. The aim is to produce effective vaccines that are safe for a civilian population that is widely diverse with respect to age and state of health. NIAID's strategy for vaccine development is to optimize our current capabilities in the near term and create safer, more effective vaccines over the long term.

The approach to smallpox vaccine illustrates this paradigm. Recently, NIAID smallpox vaccine dilution studies have provided valuable information about the potential use of our vaccine (Dryvax). These studies have shown that if necessary, the current stockpiles of Dryvax can be diluted fivefold or even tenfold to stretch the limited vaccine supply, while retaining adequate immunogenicity. By the end of 2002, more than 200 million new doses of smallpox vaccine derived in cell culture will be produced, tested, and stockpiled. Researchers already are designing third-generation vaccines to be more immuno-

Instinct and Commitments to Public Service Helped Fauci Make Key Choices

Anthony S. Fauci is running out of room for displaying his awards, even in the spacious office allotted him as director of the National Institute for Allergy and Infectious Diseases (NIAID). With plaques already spilling into an outer office, the placement of his latest certificate, which accompanied the $500,000 Albany Medical Center Prize in Medicine and Biomedical Research last April, may be a challenge. The certificate cites his seminal contributions to HIV/AIDS research and his dedication to public service.

While awards consume one wall of Fauci's office, dozens of framed portraits, including Fauci with George and Barbara Bush, the Clintons, and Elizabeth Taylor, adorn another. Nearby, scores of snapshots capture moments shared with his wife, three daughters, friends, colleagues, and patients. Other photos depict him in his seldom-indulged pastime of fishing; in one, he proudly displays a catch that's half his height.

The items on these walls represent key forces behind Fauci's career: hard work, public service, and commitment to family, colleagues, and patients. Some of the credit behind his success goes to a demand for excellence, drive for perfection, and work ethic that routinely bring him to his office by 7 AM, even on Saturdays. He also credits his upbringing and education at Jesuit institutions for instilling a dedication to public service. In addition, he is unafraid to choose an unpopular path, even in the face of vociferous criticism. "A lot of it is instinctual," he says. "Your instincts tell you that you should be pursuing this or not pursuing that."

In the early 1980s, when AIDS had not yet been named, Fauci shifted his research focus to this little-known disease that then affected only a relatively few gay men and intravenous drug users. Even now, he is not sure how or just why he chose to do so. "It isn't like I'm smarter than a lot of smart people out there," he insists. "It was just an intuitive feeling." At first, this interest struck colleagues as odd, if not downright self-destructive. "I was in fact criticized by some friends who said, 'You're going to be throwing away a career that's accelerating to chase a disease that doesn't even have a name!' But I said no, there's something about this that feels right."

Fauci again followed his intuition when he proposed a substantial budget boost for HIV research after becoming director of NIAID in 1984.

"There was a tremendous degree of anxiety among some of the older, established infectious disease people that this young whippersnapper was going to start pursuing this strange, new disease...and he's going to wind up having money siphoned away from other established programs," Fauci recalls. "Here I am, a 45-year-old new director and these guys who've been doing it 20 years longer than I have are saying don't do it. So I thought about it a lot and I said. . . 'This is going to be a catastrophic epidemic and how would I look myself in the mirror 10 years from now if we lost time?'"

NIAID received extra funding for HIV research, and its budgets continue to expand. Since 1984, the institute has grown from the eighth largest at NIH to become the second largest, with an annual budget of almost $4 billion.

Fauci gambled again in the late 1980s when he endorsed parallel tracking in clinical trials, the practice of providing HIV patients who were not enrolled in trials

Continued

genic, less reactogenic, and safer for use by all populations. Thus, the smallpox vaccine research and development objectives will be completed in stages, over several years.

Other vaccines are nearing clinical trials, such as an anthrax vaccine based on recombinant protective antigen (rPA) and an Ebola virus vaccine that has already proven to be effective in a nonhuman primate model.

• **Therapeutics.** Advances in developing new therapeutics depend heavily on progress made in the areas of microbial biology and host defenses. However, rational screening of existing antimicrobial compounds has already begun to yield useful results. In collaboration with the Department of Defense and the CDC, NIAID has been screening antiviral drugs for activity against orthopoxviruses. The discovery two years ago that

early access to experimental drugs. "Back then that was anathema," he says. "You can't imagine how many people said, 'You're going to be responsible for destroying the integrity of clinical trials.'" Invited by leading AIDS activists to speak at a meeting in San Francisco, Fauci told no one in advance of this new program. "When I walked onto that stage to give the announcement that I was endorsing parallel tracking, I said, 'I might have to find another job tomorrow, but here it goes!' But there was no doubt in my mind that . . . it was the right thing to do.

"As it turned out, once I broke the ice and said we should really seriously consider parallel tracking, very quickly the FDA and the Department of Health and Human Services came out and said, 'You know, that's not such a bad idea; we endorse it.' So it was something I thought I was going to have to explain to everyone from the White House to the Secretary of Health and Human Services, and they wound up giving me a medal for it." Fauci quickly qualifies this follow-your-gut account of his decision-making by emphasizing the importance of including a thorough analysis of any situation. "All those decisions were not made by the seat of my pants," he says. "They were made with a combination of instinct and a lot of good due diligence."

Some of Fauci's success derives from his knack for connecting with people, be they politicians who determine NIH budgets, a worried public facing an anthrax scare, or angry AIDS patients lashing out at the health care system. Early during the AIDS epidemic, Fauci found himself vilified as a "monster" and hung in effigy. The attacks even extended to his family, with activist leader and ACT-UP cofounder Larry Kramer also criticizing Fauci's wife Christine Grady, who is a bioethicist at NIH.

In dealing with such criticisms, Fauci takes to heart the credo in *Harrisons's Principles of Internal Medicine*: "To the physician as to the anthropologist, nothing human is strange or repulsive....The true physician has a Shakespearean breadth of interests in the wise and the foolish, the proud and the humble, the stoic hero and the whining rogue. He cares for people."

"That's a phenomenal paragraph," Fauci says. "I think being a physician and realizing that these are sick people who are frightened, who are angry, who feel hopeless—they're trying to get my attention so they're throwing these incredible barbs at me to be hurtful because when you're dying, you're lashing out, you're afraid. So . . . I would rather turn the other cheek. . . ." Thus, when hundreds of protesters stormed the NIH campus in 1990, Fauci invited the leaders inside to talk, touching off interactions that eventually led to his support of parallel tracking. Kramer, once a relentless critic, has become both a patient and friend, dining at the Fauci home, where he apologized to Christine with roses.

Last year, Fauci helped the Bush administration in its handling of the anthrax scare, and he is credited for coolly providing realistic but reassuring information during a period of confusion, rumors, and fear. "People really want to understand the rationale behind public health decision-making," he says. "You give them the explanation, they may agree with you, they may disagree with you, but they will respect you for giving them the information."

At 62, Fauci still exhibits youthful enthusiasm and energy. "I think I'm at a very productive stage of my career and a very energetic leadership position," he says. "If those ever start to slip even a little, I will certainly look for something else to do. This is a case where I would listen to myself, and I have a very good radar for things like that."

Christine Stencel
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cidofovir protected mice from aerosol challenge with cowpox was followed this March by further progress. NIAID-supported researchers discovered an oral derivative of cidofovir whose efficacy against cowpox in vitro suggests that it is possible to develop an oral formulation superior to the parenterally administered product.

Antitoxins, immunotherapeutics, and novel antimicrobial drugs are also being intensively studied; their development relies heavily on an appreciation of the pathogenic mechanisms of microbial disease. Research supported in part by NIAID has led to the development of an anthrax antitoxin that exploits a dominant-negative mutant PA to block translocation of other *B. anthracis* toxins into host cells. The Department of Defense is pursuing animal studies of the antitoxin, which is a potential adjunct to standard

therapy of anthrax. Existing immune-based therapies, such as disease-specific immune globulins, are being produced and evaluated. An eventual goal is the development of a panel of human monoclonal antibodies against immunogenic epitopes of every major pathogen.

● **Diagnostics.** Great strides have been made—and remain to be made—in the area of diagnostics for microbes that might be used in a bioterrorist attack. NIAID-supported researchers and their collaborators are creating an array of rapid diagnostic tests for clinical use. They are following many existing diagnostics paradigms, including antigen capture, serology, and restriction fragment-length polymorphism (RFLP) analysis, as well as using new technologies such as microarrays, to develop novel diagnostic tests. The goal is to establish and validate rapid, sensitive, and specific tests that can detect multiple microbial strains or toxin serotypes, including those that have been bioengineered. In an outbreak with an unknown cause, clinicians would need these diagnostic tests to identify pathogens and determine their sensitivity to existing antimicrobial agents.

● **Research Resources.** Infrastructural deficiencies are a constant problem in the biomedical sciences, as ideas and technology outpace facilities and other shared resources. Biodefense research requires specialized resources. Any comprehensive study of highly infectious microbes and development of protective strategies requires the availability of high-containment laboratories capable of conducting aerosol challenge; centralized databases for research on genomics and proteomics; standardized reagents; animal models, particularly nonhuman primates; and clinical trials capacity. NIAID is working to meet each of these objectives so that biodefense research and development can proceed at an accelerated pace. The Institute will soon begin the renovation and construction of BSL-3 and BSL-4 labs in order to expand national high-containment capacity. Another significant initiative is the establishment of the first four to seven of 10 planned Regional Centers of Excellence for Biodefense and Emerging Diseases Research, where specialized resources and expertise will fuel basic and applied research on potential bioterror agents and other emerging and re-emerging pathogens.

NIH plan for biodefense research, FY 2003

Program	Funding
Research facilities construction	$521.1 million
● Build BSL3 and BSL4 labs (including at the comprehensive research centers)	
Basic research on agents of bioterrorism	$440.6 million
● Support research programs in at least four comprehensive extramural centers	
● Conduct genomic sequencing and proteomic analysis on up to 25 pathogens	
● Expand training programs	
Drug/vaccine/diagnostics discovery and development	$591.9
● Test and develop candidates for next-generation anthrax vaccine	
● Engage industry through challenge grants	
● Establish repositories for diagnostic and drug reagents	
● Develop animal models, establish high-containment facilities and services	
Clinical research	$194.3 million
● Expand clinical trials infrastructure (VTEUs)	
● Conduct smallpox, anthrax, and Ebola clinical trials	
TOTAL	$1,747.9 million

Spinoffs for Other Fields

Aggressive expansion of biodefense research and development targets will inevitably have unforeseen, beneficial effects on other areas of basic science and medicine. New knowledge of microbial biology and host defenses may reinvigorate other fields and produce useful therapies. For example, better understanding of the mechanisms of regulation of immune responses as well as the pathways of host defenses against exogenous or endogenous invaders will have important implications for research in the fields of cancer and immune-mediated diseases. Furthermore, novel vaccine, diagnostic, and therapeutic strategies may help to reduce the global burden of important infectious diseases such as HIV/AIDS, malaria, and tuberculosis.

There are many examples of such spinoffs in science and medicine. Some of many clear-cut examples are seen from the large investment of resources in HIV/AIDS research. Antiviral drug discovery for HIV/AIDS produced two effective treatments for hepatitis B (lamivudine and adefovir). Ganciclovir, developed for HIV-associated cytomegalovirus (CMV) infection, is now used routinely for prophylaxis in transplant pa-

tients. Cidofovir, developed for CMV infection in patients with advanced HIV disease, has considerable activity against poxviruses in animal models and may be effective against smallpox or against the complications of vaccinia vaccine. HIV-oriented research on retroviruses has led to the use of retroviral vectors in experimental cancer vaccines and gene therapy.

Challenges

Biodefense research agendas at NIH and other institutions face several common challenges, including a shortage of trained scientists and research resources. If researchers are successful, the development and delivery of pharmaceutical or biological products for biodefensive or other use will require corporate partners willing to manufacture them. Finally, the anthrax attacks and subsequent investigations have raised doubts about the security of potential pathogens in research laboratories as well as the accessibility of information related to these pathogens. The following challenges may have major implications for the way in which we conduct collaborative science.

● **Expanding the community of biodefense scientists.** There is a dearth of investigators involved in research on some of the most important pathogens that could potentially be used in a bioterrorist attack. For example, relatively few bench scientists in the country are currently working on the bacterium that causes tularemia, *Francisella tularensis*, one of the six Class A agents.

There are several reasons behind the shortage of biodefense research personnel. First, substantial research funding targeted to these pathogens became available only in the past 5 years. Relatively little attention was paid to these uncommon infections until the mid-1990s, when officials became concerned about the possible dispersal of biological agents from the former Soviet Union weapons program. Prior to that, greater funding urgency was deservedly assigned to widespread emerging infections, such as HIV/AIDS, tuberculosis, and malaria. The number of Americans living with HIV/AIDS is approaching 1 million; the number reported to be infected with plague in 1999 was only 9.

Second, the severely limited national capacity for high-containment laboratory work may have turned many investigators away from pursuing research in this area. Such significant infrastructural deficiencies are bound to discourage prospective investigators.

Finally, the difficulties of collaboration and sharing of resources among scientists who are spread far and wide may deter some from research on unusual pathogens. The planned Regional Centers of Excellence in Biodefense and Emerging Diseases Research are intended to eliminate some of the logistical obstacles to teamwork among biodefense investigators and contribute to the local, state, and national biodefense infrastructures. The Centers will aim to attract scientists from fields such as immunology, microbiology, and virology to work together in biodefense research. A multidisciplinary approach to biodefense research—especially the study of microbial biology and host defenses—will likely yield knowledge and products useful in combating bioweapons as well as other human illnesses.

● **Infrastructure.** The biodefense research community finds itself, in many instances, to be lacking needed research infrastructure. There is a serious shortage of high-containment laboratories in which to perform experiments using dangerous pathogens; animals, particularly nonhuman primates, with which to conduct such studies; and standard reagents. NIAID's planned construction of BSL-3 and BSL-4 facilities over the next four years will greatly expand national capacity to perform research on the highly infectious agents whose deliberate release could cause widespread harm. The ability to conduct aerosol challenge using small animals and nonhuman primates will be essential for testing drugs and vaccines and for establishing correlates of immunity. In addition to specialized laboratory space, nonhuman primates for research have been scarce, in part because of high international demand. For many pathogens, nonhuman primates are the most relevant animal models, and are therefore needed for safety evaluations, challenge studies, and validation of immunologic assays.

● **Industry involvement.** A significant challenge in the development and manufacturing of drug, biologic, and diagnostic products is the need for partnership with industry. A sense of responsibility for the common good has not been sufficient to drive private sector research and devel-

opment on vaccines and drugs in an era of high costs. There are inadequate incentives for the biotechnology and pharmaceutical sectors to invest resources in developing tools that may never be used, and whose production may never be renewed. The current shortage of vaccines has demonstrated that, in the current market, even the production of vaccines with widespread use is endangered. A new paradigm of partnership with the biotechnology and pharmaceutical industries will be essential to promote the development and manufacturing of the biodefense armamentarium. We must establish the interest and motivation to engage the private sector in the national mission of biodefense. This can be done if we are deliberate and deliberative. We must partner with the private sector in the development and production of these products that enhance our leadership in biotechnology. We need to maintain this leadership not only to protect ourselves from future attacks should they occur, but most importantly to serve as a deterrent against the launching of such attacks.

• **Biosecurity.** Much of what has been written above relates to efforts designed to enhance our defense against the potential of a biological attack. Equally important is the concept of "Biosecurity." Biosecurity can be defined as the set of activities designed to enhance the physical security of laboratories and their contents, especially laboratories in which research on so-called "select agents" is being conducted.

Most of this is aimed at protecting microbiologists. Laboratory work with these infectious agents has always presented increased hazards to those engaged in the work. It is essential that we provide the maximum amount of physical protection to the microbiologists who work in these environments and to the communities surrounding these facilities. This means that we must be assiduous in monitoring where these organisms are being studied.

The question of data access is also an increasing source of debate. Should we limit the distribution of or classify some of the research being performed on these pathogens? Is shared knowledge, in itself, an important and useful deterrent? Clearly, the threat of additional attacks will be with us for many years, and the microbiology community must play a leadership role in the national effort to enhance biosecurity and in the discussion of issues related to data access.

Public Health Questions

The current debate over whether to vaccinate the U.S. population against smallpox highlights important challenges in public health decision-making with regard to biodefensive vaccines and therapeutics. Public health authorities and policymakers will need to make difficult determinations of how and when vaccines should be distributed. There is no generic plan applicable to any attack; responses must be tailored to agents involved, site(s) of release, and groups affected. A major challenge is that any plan must include strategies for protecting young children, pregnant women, the elderly, and the immunocompromised.

Once a panel of vaccines against potential agents of biowarfare is available, we may face dilemmas similar to the current one involving the smallpox vaccine. The recent vaccine dilution study and the likely contribution of more than 75 million doses of smallpox vaccine by Aventis-Pasteur, together with the ongoing production of at least 155 million doses of vaccine by Acambis/Baxter, have opened a discussion of the appropriateness of mass vaccination or voluntary vaccination against smallpox. Now that it is clear that there will soon be potentially enough vaccine to inoculate the entire country, should we then use it? Those who point to the high rate of vaccine complications in the 1960s and the lack of evidence of imminent release are countered by others who see vaccination as a safety measure and a deterrent. This divergence of opinions has been worthy of an open discussion and debate, as it may be a basis for future deliberations regarding use of other biodefensive vaccines.

Conclusion

The challenge of biological terror will be with us for many years. The renewed emphasis on research into infectious diseases, especially emerging infectious diseases, and into the host responses to those pathogens, will produce enormous potential benefits for humankind. Vast new knowledge about the microbial world and the human response to infection will cer-

tainly be discovered by this massive research effort.

In fact, it is appropriate to view biodefense research within the umbrella of continuing research on emerging and reemerging infectious diseases. Emergence and reemergence of pathogens generally occur as part of a natural evolution of interactions between the human species and the vast array of microbes. Bioterrorism is merely the deliberate release of microbes that by definition reemerge within a unique set of circumstances. In either case, it is the responsibility of the biomedical research and public health communities to provide the necessary tools to serve the defense of the nation, the world, and the public health.

SUGGESTED READING

Bradley, K. A., et al. 2001. Identification of the cellular receptor for anthrax toxin. Nature **414:**225–229.
Bray, M., et al. 2000. Cidofovir protects mice against lethal aerosol or intranasal cowpox virus challenge. J. Infect. Dis. **181:**10–19.
Centers for Disease Control and Prevention. 2001. Summary of notifiable diseases, United States, 1999. Morbid. Mortal Weekly Rep. **48:**1–101.
Central Intelligence Agency. Unclassified report to Congress on the acquisition of technology relating to weapons of mass destruction and advanced conventional munitions, 1 January through 30 June 2001. Langley, Va., Central Intelligence Agency, January 30, 2002.
Fauci, A. S. 2002. Smallpox vaccination policy – the need for dialogue. New Engl. J. Med. **346:**1319–1320.
Henderson, D. A. 1999. The looming threat of bioterrorism. Science **283:**1279–1282.
Lane, H. C., J. La Montagne, and A. S. Fauci. 2001. Bioterrorism: a clear and present danger. Nature Med. **7:**1271–1273.
Monterey Institute of International Studies. 2002. Chemical and biological weapons: possession and programs past and present. Monterey Institute of International Studies, Monterey, Calif.
Sellman, B. R., M. Mourez, and R. J. Collier. 2001. Dominant-negative mutants of a toxin subunit: an approach to therapy of anthrax. Science **292:**695–697.
Sullivan, N. J., A. Sanchez, P. E. Rollin, Z. Y. Yang, and G. J. Nabel. 2000. Development of a preventive vaccine for Ebola virus infection in primates. Nature **408:** 605–609.