The anthrax attacks in the United States, juxtaposed against the September 11 terrorist attacks on New York and Washington, DC, have transformed a theoretical threat to stark reality. The biomedical research community will be an integral part of the preparation for, defense against and response to bioterrorism

Bioterrorism: A clear and present danger

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The terrorist attacks of 11 September took the lives of approximately 5,000 innocent individuals, destroyed the World Trade Center in New York City, severely damaged the Pentagon in Washington, DC, and led to the death of the crew and passengers of 4 commercial airliners. This tragedy will transform society in the United States and worldwide in ways that we are only now beginning to discern. The weeks following that tragedy have been marked by vastly increased security measures and deep concern about the possibility of additional attacks. Sadly, the fears of additional attacks were realized on 5 October when the first in a series of anthrax cases was reported from Boca Raton, Florida[1,2], and later from Trenton, New Jersey, Washington, DC, and New York City[3]. To date a total of 17 cases of anthrax have been confirmed and 5 cases of cutaneous disease have been classified as suspected. Ten of the confirmed cases were inhalation anthrax[4] while the remaining 7 were cutaneous anthrax. Four people have died. More cases may be identified as the investigation continues. More than 30,000 people have been placed on prophylactic antibiotics, a strategy that carries its own risk[3]. In addition to the direct human toll of cases and deaths, there are additional expenses such as the decontamination of many public buildings. The prospect of anthrax as a weapon of war is not new[5], but this is the first time that anthrax has been deliberately used in this manner and it is not likely to be the last. It is unclear whether the events of 11 September and the anthrax attacks were perpetrated by the same group of terrorists or whether the latter was the work of a domestic terrorist group or a single deranged individual. However, their proximity in time links them irrevocably in both the public perception and the impetus to fight terrorism.

Bioterrorism versus biowarfare

The civilian population in the US may never be able to return to that state of false security, of believing that some acts are so repugnant that they are beyond human deed. A sense of complacency and safety is now replaced by a sense of unyielding urgency. The theoretical has become a reality. The relatively brief moment of respite from anxiety afforded by the end of the Cold War has been replaced by the realization that there are people who would stop at absolutely nothing to inflict pain, terror and death upon their fellow humans, including ordinary citizens. Terrorist acts generate fear and panic through their utterly unpredictable nature; the next attack can happen anywhere at anytime with any one of a number of weapons. In this and other respects, bioweapon attacks against the civilian population are different from those against military targets. Civilian populations are much more vulnerable to bioweapons in that they represent far more diverse ages and states of health. Also, military personnel are prophylactically vaccinated whereas unexpected civilian attacks—as with the recent anthrax mailings—will require rapid diagnosis and antimicrobial treatments wherever appropriate and available. Finally, the potential agents and circumstances of bioweapon attacks in civilian settings are more diverse than those directed at the military; attacks against civilians are usually intended to cause widespread panic and terror.

Preparation for bioterrorist attacks against civilians takes two major forms: 1) intelligence and law enforcement activities to prevent attacks, and 2) public health activities to prepare for, respond to and lessen the impact of attacks. With regard to the latter, the preparation for and response to bioterrorism must be multifaceted and comprehensive. It must employ classic public health preparedness and activities at the federal and local levels. These include revitalizing the capacity of local public health facilities, training of teams of 'first responders', developing and updating plans and guidelines for immediate responses at the local level, and providing for the availability of vaccines, antibiotics and other medical supplies for emergency deployment. The role of the scientific community in preparedness for and response to bioterrorism is no less important, for it is the community of biomedical researchers who have been providing and will continue to provide the knowledge base that will ultimately be translated into effective tools in this comprehensive team effort. Among these tools will be the vaccines, drugs and diagnostic tests that will be critical to diminish the threat of bioterrorism.

The research agenda

The recent anthrax attacks have revealed gaps in our knowledge that compromise our ability to respond to a bioterrorist attack. The appropriate antibiotic drugs to use and the duration of treatment remain uncertain, as does the role of the existing anthrax vaccine in the setting of post-exposure treatment[6,7]. These two examples illustrate some of the key questions that will need to be addressed if the responses to future anthrax attacks are to be most effective. Only by engaging the full range of capabilities in the biomedical research community and allying these to effective intelligence gathering efforts and law enforcement activities will we be able to effectively counter the real threats presented by bioterrorism.

Research on the potential agents of bioterrorism has been a priority for several years at the National Institutes of Health (NIH) as part of a broader program at the Department of Health and Human Services that includes efforts by the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA) and the Office of Emergency Preparedness (OEP). Fig. 1 summarizes the increasing support for research on the biological agents of bioterrorism by the NIH over the past five years. Appropriations for the NIH bioterrorism program are projected to increase substantially in the immediate future and it is likely that this will take the form of additional as opposed to redirected resources. The present research effort is heavily focused on basic research into the pathogens that are acknowledged to be genuine bioterrorism threats. Programs for the development of diagnostics, drug discovery, vaccine development, clinical research and epidemiology will complement basic research. In order to focus attention on those agents most likely to cause the greatest harm in a bioterrorist attack, a listing of 'select agents' has been compiled by the CDC. This listing of select agents separates potential agents of bioterrorism into three categories according to risk,

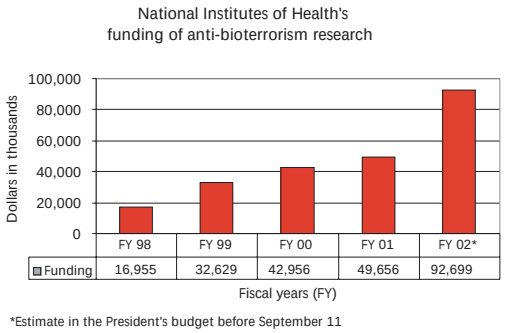

Fig. 1 NIH budgets for anti-bioterrorism research, 1992–2002.

A–C (Table 1) (http://www.bt.cdc.gov/Agent/Agentlist.asp). The highest priority at present is support for research on the agents identified in category A, especially smallpox and anthrax.

Smallpox

As a result of aggressive global vaccination programs, smallpox was eradicated from the world as a naturally occurring disease in 1977 (ref. 8). Consequently, routine vaccinations against smallpox were discontinued in the US in 1972 and in the rest of the world by 1979. Thus, in 2001, with few exceptions, the world's population is either immunologically naive to smallpox or possesses diminished immunity as a result of vaccinations that were administered decades ago. Legal and recognized laboratory stocks of the virus (variola major) have been retained in at least two locations—the US and Russia. However, defectors from the bioweapons program of the former Soviet Union describe how smallpox for use as a bioweapon was produced in large amounts in the 1970s in the Soviet Union[9]. It is uncertain whether some of this material could have been acquired by potential bioterrorists. Clearly, smallpox as a biological weapon is a real threat.

Although emergency vaccine supplies are available, the vaccine has been out of production in the US for over 30 years. Studies are currently underway to determine whether the US government's reserve of 15 million doses of the established Dryvax vaccinia vaccine produced *in vivo* in calf lymph can be diluted and extended to 75 or 150 million doses. Additional supplies of second-generation vaccine that are produced in tissue culture and that are safer to use are urgently needed[10,11]. This need poses some immediate research challenges, particularly with regard to the determination of efficacy in the absence of naturally occurring disease and the question of safety in infants, pregnant women and individuals with various immunodeficiencies including HIV infection and iatrogenic immunosuppression. To minimize the impact of vaccinia reactions in immunocompromised individuals, it is critical to re-establish the supply of vaccinia immune globulin as well as develop alternatives such as humanized monoclonal antibodies. Modern molecular biological techniques must be applied to the rapid diagnosis of smallpox, an asset that will be critical to the early containment of an outbreak.

There is currently no proven, effective specific therapy for smallpox. However, certain studies in animal models of poxvirus infection are encouraging. For example, certain nucleoside or nucleotide analogs, such as cidofovir, appear to be effective against orthopox viruses including variola major and vaccinia[12,13] . Additional drugs are being sought through drug-screening programs and rational drug discovery efforts that rely on the new information derived from genomic sequencing. The genome sequences of several variola strains, as well as other orthopox viruses, have been derived[14] and are serving as a valuable research resource for the discovery and development of new vaccines, drugs and diagnostic tests.

Anthrax

Anthrax is an ancient and familiar disease whose pathogenesis is well understood[15]. There is an FDA-approved vaccine for anthrax called 'anthrax vaccine adsorbed' (AVA) that has been used since 1970 in the US military and in individuals with occupational risk for anthrax. It is a vaccine derived from culture supernatant that has proven to be effective, but has been troubled with production problems and at least the perception of significant toxicities. Pathogenic variants of *Bacillus anthracis* contain two plasmids that play a major role in the disease-producing potential of the organism. One of these encodes the proteins involved in the D-glutamic-acid capsule. This capsule is thought to render the organism resistant to phagocytosis. The other plasmid codes for the three proteins: lethal factor (LF), edema factor (EF) and protective antigen (PA); these are involved in the creation of two known toxins: lethal toxin (LT) and edema toxin (ET). To exert their effect, either factor must combine with the cleaved form of PA (ref. 16). Thus, PA is a key element in the pathogenesis of anthrax. This protein assembles into a heptamer that is required for transposition of either LF or EF into the cytoplasm. These two toxin factors, EF and LF, share the same receptor on the PA heptamer and antibodies specific for PA neutralize the toxic effects of either LF or EF (ref. 17).

At least three new anthrax vaccines based on the PA protein are under development or study[18–20]. This protein can be produced in large amounts and clinical trials are expected to begin in early 2002. PA is also a promising target for development of therapeutic drugs. Dominant mutants of the gene encoding PA have been shown to produce a mutant PA protein that inactivates the as-

Table 1 Varying threat of select infectious agents

Category A	Category B	Category C
Bacillus anthracis (anthrax)	*Coxiella burnetti* (Q fever)	Nipah virus
Clostridium botulinum toxin (botulism)	*Brucella* species (brucellosis)	Hantaviruses
Yersinia pestis (plague)	*Burkholderia mallei* (glanders)	Tickborne encephalitis viruses
Variola major (smallpox)	Ricin toxin from *Ricinus communis*	Yellow fever
rancisella tularensis (tularemia)	Epsilon toxin of *Clostridium perfringens*	Multidrug-resistant tuberculosis
Viral hemorrhagic fevers	*Staphylococcus* enterotoxin B	

Category A agents are those that can be easily disseminated or transmitted person-to-person; cause high mortality, with potential for major public health impact; might cause public panic and social disruption; and require special action for public health preparedness. Category B agents are next in priority and include those that are moderately easy to disseminate; cause moderate morbidity and low mortality; and require specific enhancements of the CDC's diagnostic capacity and enhanced disease surveillance. Category C consists of emerging pathogens that could be engineered for mass dissemination in the future because of their availability, ease of production and dissemination, and potential for high morbidity and mortality.

sembled heptameric PA (ref. 21). Antibody-based therapies for anthrax have also been proposed and are under development. The use of antibody-based therapies or vaccination in the immediate post-exposure setting may allow for a decrease in the duration of the current 60-day regimens of antibiotics presently recommended[6]. These agents also have the theoretical possibility of reversing attachment of PA to the cellular receptor. Finally, the two toxins may also be targets for therapeutic intervention through rational drug discovery efforts. One of these toxins, LF, is known to be a zinc-metalloprotease and is thought to be a specific target for the development of protease inhibitor therapies[22]. The recent determination of crystalline structure of LF should facilitate this avenue of research[23].

Other priorities

The research agenda includes other priorities. Candidate DNA and adenoviral-vector-based vaccines to prevent Ebola virus infections are under development[24]. Expanded efforts in genomic sequencing and informatics also need to be emphasized. The information generated from these activities will yield important clues for drug, vaccine and diagnostic test development. This information can also be of great value in studying the molecular epidemiology of infectious agents. The sharing of data and the development of consolidated data sets with genomic information will be of value in disease surveillance and will help guide responses to bioterrorist attack.

Bioterrorism and the biomedical research community

Bioterrorism is now an important part of the research agenda for the biomedical research community. It must be given a status similar to that of research in other pressing areas such as malaria, tuberculosis and AIDS. Recent events have made it clear that what was once a theoretical concern is now a clear and present danger. What began as an area of boutique research—at times regarded with skepticism by mainstream science—is now the center of attention for the biomedical research community. But research on microbes of bioterrorism should not be viewed in a vacuum; rather, we should consider these investigations to be critical components of the broader arena of research on naturally emerging and re-emerging microbes. Bioterrorism is in fact the deliberate, planned and unnatural re-emergence of pathogenic microbes in settings designed to cause maximal suffering and death.

The recent bioterrorism events have presented the biomedical research community with some new responsibilities. One of the most important will be educational, of actively informing the public and policymakers about the nature of the threats that confront us in an accurate and realistic manner. There is also an acute need to expand training for established scientists through national and international professional societies and organizations, and to develop training pathways for new scientists. We will need to develop new strategies for coordinating efforts across disciplines, such as environmental health and molecular biology, across agencies, such as defense and health, and across nations.

The biomedical research community must be viewed as being in the front line of a war in which a primary weapon has been the evil application of biomedical technology. Common goals rather than individual achievements must be the rule—the primary motivation for excelling in this area of research must be the protection of our society. A tragic possibility is that the perpetrators of the most recent events in the US may have come from our own ranks: Someone in the biomedical research community might be responsible for the deliberate release of anthrax spores, the murder of at least four innocent individuals, the imparting of pain and suffering on several others, and the serious disruption of society. Recent events also suggest that there may be a need for more restrictions on access to certain materials that can be used for illegal purposes. Surely, well-intentioned legislators will consider, and appropriately so, the enactment of laws to protect society against bioterrorist threats. The scientific community must take the leadership role in assisting lawmakers in determining the correct balance between academic freedom and public safety.

The ultimate goal of biomedical research is to advance the public health of society. In this regard, the biomedical research community clearly has a responsibility to participate in the current and future struggle against bioterrorism. Today's investments in research must take into account today's exigencies. This will be an enduring effort and we must make enduring commitments.

1. Anthrax case confirmed 63-year-old man hospitalized. The Associated Press. October 4, 2001.
2. Ongoing investigation of anthrax—Florida, October 2001. *MMWR Morb. Mortal. Wkly. Rep.* **50**, 877 (2001).
3. Update: Investigation of bioterrorism-related anthrax and adverse events from antimicrobial prophylaxis. *MMWR Morb. Mortal. Wkly. Rep.* **50**, 973–976 (2001).
4. Jernigan, J.A. *et al.* Bioterrorism-related inhalation anthrax: The first 10 cases reported in the United States. *Emerg. Infect. Dis.* posted on-line 8 November 2001.(www.cdc.govlncidodleid/vol7nos6/pdf/jcrnigan).
5. Inglesby, T.V. *et al.* Anthrax as a biological weapon: medical and public health management. Working Group on Civilian Biodefense. *JAMA* **281**, 1735–1745 (1999).
6. Friedlander, A.M. *et al.* Postexposure prophylaxis against experimental inhalation anthrax. *J. Inf. Dis.* **167**, 1239–1242 (1993).
7. Use of anthrax vaccine in the United States. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb. Mortal. Wkly. Rep.* **49**, RR-15 (2000).
8. Henderson, D.A. Principles and lessons from the smallpox eradication programme. *Bull. World Health Organ.* **65**, 535–546 (1987).
9. Alibek, K. & Handelman, S. *Biohazard: The Chilling Story of the Largest Covert Biological Weapons Program in the World—Told from Inside by the Man Who Ran It.* (Dell, New York, New York, USA, 1999).
10. LeDuc, J.W. & Becher, J. Current status of smallpox vaccine. *Emerg. Infect. Dis.* **5**, 593–594 (1999).
11. Andzhaparidze, O.G. *et al.* Investigation of tissue culture MNIIVP smallpox vaccine in a coded controlled trial by revaccination of adults by scarification. *Vopr. Virusol.* **4**, 443–446 (1980).
12. De Clercq, E. Vaccinia virus inhibitors as a paradigm for the chemotherapy of poxvirus infections. *Clin. Microbiol. Rev.* **14**, 382–397 (2001).
13. Bray, M. *et al.* Cidofovir protects mice against lethal aerosol or intranasal cowpox virus challenge. *J. Inf. Dis.* **181**, 10–19 (2000).
14. Analysis of the nucleotide sequences of variola virus genomes. *Bull. World Health Organ.* **72**, 813–814, 817–818 (1994).
15. Dixon, T.C., Meselson, M., Guillemin, J. & Hanna, P.C. Anthrax. *N. Engl. J. Med.* **341**, 815–826 (1999).
16. Singh, Y., Chaudhary, V.K. & Leppla, S.H. A deleted variant of *Bacillus anthracis* protective antigen is non-toxic and blocks anthrax toxin action *in vivo*. *J. Biol. Chem.* **264**, 19103–19107 (1989).
17. Reuveny, S. *et al.* Search for correlates of protective immunity conferred by anthrax vaccine. *Infect. Immun.* **69**, 2888–2893 (2001).
18. Friedlander, A.M., Pittman, P.R. & Parker, G.W. Anthrax vaccine: evidence for safety and efficacy against inhalational anthrax. *JAMA* **282**, 2104–2106 (1999).
19. Thomas, L.J. *et al.* An immunogenicity and reactogenicity evaluation of a recombinant *Bacillus anthracis* protective antigen vaccine. 4th International Conference on Anthrax. *Abstracts Book* June 10–13, 2001, Annapolis, Maryland, USA.
20. Turnbull, P.C.B. Current status of immunization against anthrax: old vaccines may be here to stay for a while. *Curr. Opin. Infect. Dis.* **13**, 113–120 (2000).
21. Singh, Y., Khanna, H., Chopra, A.P. & Mehra, V. Dominant negative mutant of *Bacillus anthracis* protective antigen inhibits anthrax toxin action *in vivo*. *J. Biol. Chem.* **276**, 22090–22094 (2001).
22. Klimpel, K.R., Arora, N. & Leppla, S.H. Anthrax toxin lethal factor contains a zinc metalloprotease consensus sequence which is required for lethal toxin activity. *Mol. Microbiol.* **13**, 1093–1100 (1994).
23. Pannifer, A.D. *et al.* Crystal structure of the anthrax lethal factor. *Nature* **414**, 229–233 (2001)
24. Sullivan, N.J., Sanchez, A., Rollin, P.E., Yang, Z.Y. & Nabel, G.J. Development of a preventive vaccine for Ebola virus infection in primates. *Nature* **408**, 605–609 (2000).

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