The Labyrinth of Biological Defense

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The history and content of the United States Army's Biological Defense Research Program are outlined. Evidence is given that current research is not limited to drugs, vaccines, detection devices, and protective gear, as claimed, but may extend to potentially more offensive applications, such as production of toxins, more virulent strains, and improved methods of delivery of biological agents. These research activities may arguably place the U. S. Army's Biological Defense Research Program in violation of U. S. government policy and the Biological Weapons Convention. PSRQ 1991;1:24–30

Should the United States be prepared to defend itself against a biological warfare attack? Should it prepare itself to wage biological warfare—perhaps only as a deterrent to other countries' doing so?

This question has been the source of growing controversy for the last 6 years. Funding for the Army's Biological Defense Research Program (BDRP) has increased nearly 500% in the last decade, and critics have speculated that expansion into offensive research may be fueling part of this rise [1]. This concern was reinforced by the Army's plan to build a BL-4 (maximum containment) laboratory at Dugway Proving Ground in Utah, where novel bacteria and organisms causing diseases for which there is no known cure could be tested. This laboratory was the only facility in the United States planned for aerosolized tests of pathogens. The Army's attempt to bury the funding appropriation for this laboratory in a routine request for transfer of unspent funds aroused Congressional ire and public opposition [2].

A recent response to this controversy was offered by Dr. David Huxsoll, then Director of the main laboratory for the BDRP, at Fort Detrick, Maryland. Distinguishing between offensive and defensive intent, he stated:

> The content of the program is evidence of its defensive intent: development of vaccines, drugs, diagnostic systems, and methods of rapid detection; identification of disease agents; dissemination of procedures for casualty management; and training of personnel. . . . An offensive program would include research programs on mass producing or storing large quantities of microorganisms, stabilization of microorganisms in an aerosol, on improving virulence or persistence, or on methods for dissemination and weapon development [3].

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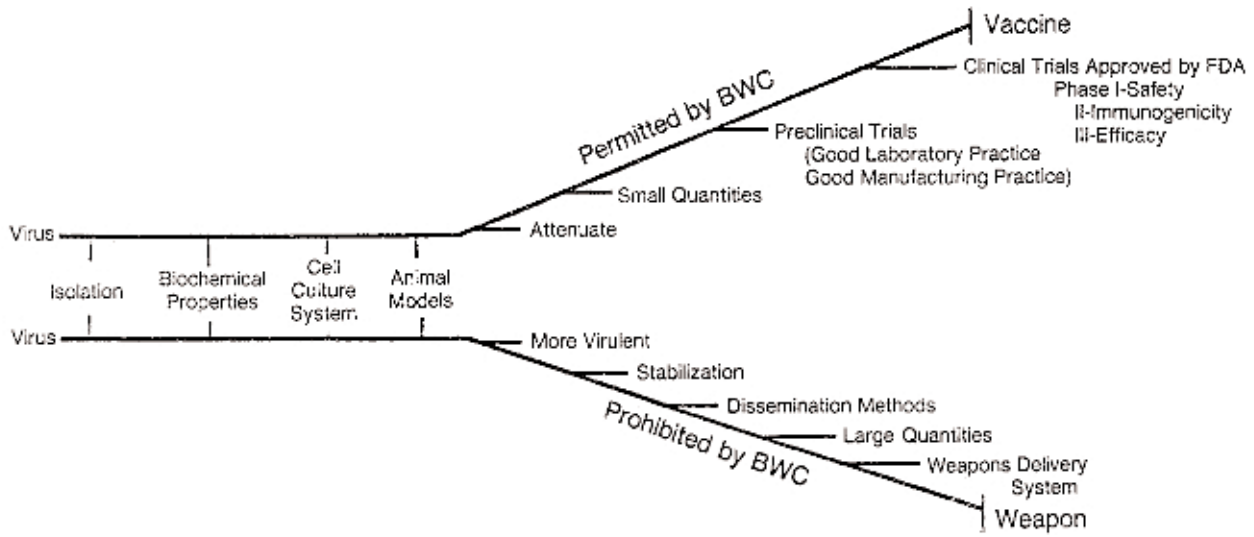

FIGURE Vaccine development and weapon development are not parallel. FDA = Food and Drug Administration; BWC = Biological Weapons Convention. Figure reproduced with permission from page 678 of Huxsoll DL, Parrot CD, Patrick, WC III. Medicine in defense against biological warfare. JAMA 1989;262(5):677–679, copyright 1989, American Medical Association.

Research pathways for defensive and offensive programs would diverge, as he outlined as reproduced in the Figure. This diagram lists the five steps needed to create a biological weapon. All of them are prohibited by the Biological Weapons Convention, according to Dr. Huxsoll, and none, according to him, are being pursued by the Army's BDRP. Yet an analysis of BDRP projects, using Dr. Huxsoll's own criteria, suggests that the Army's BDRP may be conducting biological warfare research under the guise of defense.

A HISTORICAL PERSPECTIVE

The United States first embarked on a biological warfare program in 1941, with an initial focus on the development of botulism and anthrax weapons. Twenty-eight universities received government contracts to do secret research. The United States collaborated with Great Britain on the development of anthrax bombs, and a plant was constructed near Terre Haute, Indiana with the goal of producing one million bombs per month [4]. A plan was drawn up to use anthrax bombs in an extensive air raid over six major German cities, which was to result in the death of half the population of these cities, and could have rendered each city uninhabitable for decades [5]. Before the necessary quantities of anthrax bombs were produced, however, the war ended.

During the wartime testing of anthrax weapons, the British contaminated Gruinard Island, off the Scottish coast. The island remained uninhabitable for the next 45 years. After a lengthy process of reclamation, requiring the use of defoliants and 280 tons of formaldehyde, the island was declared suitable for human habitation in 1987 [6].

At the conclusion of World War II, U.S. occupation forces learned that Japan had been engaged in an extensive program of biological weapons development [7]. The headquarters of the program was located in Pingfan, Manchuria. Between 1939 and 1940, this highly secret compound contained 150 buildings and employed 3,000 men, approximately 50 of whom were physicians.

The program developed a variety of biological agents for use as weapons, and produced large quantities of bacteria and vectors. The Japanese performed field tests on various military targets and cities in China, and 3,000 captives, including U.S., British, Australian, and Russian POWs, were used as test subjects in experiments with biological agents.

All of the Pingfan captives were killed either during the experiments or between August 10 and August 14, 1945, when the Japanese destroyed all the facilities and evacuated the Japanese staff, avoiding discovery by the advancing Soviet Army.

Lieutenant-General Shiro Ishii, a physician who was also the architect and commander of the program, was never punished, or even publicly impli-

cated for these atrocities during his lifetime, despite U.S. knowledge of his role and of his experiments on Allied POWs. Instead, the U.S. military, with General MacArthur's support, offered the Japanese scientists immunity from war crimes prosecution and the protection of on-going secrecy in exchange for information on these experiments. Three scientists from Camp Detrick (later Fort Detrick) were substantially involved in debriefing Ishii and his colleagues. In 1947, Edwin Hill, Chief of Basic Sciences at Fort Detrick, wrote the following to General Alden Waitt, Chief of the Army Chemical Corps.

> Evidence gathered in this investigation has greatly supplemented and amplified previous aspects of this field. It represents data which have been obtained by Japanese scientists at the expenditure of many millions of dollars and years of work. Information has accrued with respect to human susceptibility to those diseases as indicated by specific infectious doses of bacteria. Such information could not be obtained in our own laboratories because of scruples attached to human experimentation. These data were secured with a total outlay of Y250,000 to date, a mere pittance by comparison with the actual costs of the studies.
>
> Furthermore, the pathological material which has been collected constitutes the only material evidence of the nature of these experiments. It is hoped that individuals who voluntarily contributed this information will be spared embarrassment because of it and that every effort will be taken to prevent this information from falling into other hands [7].

Soon after, photomicrographs were made from 15,000 slides of Ishii's human pathological specimens and transferred from Japan to Fort Detrick.

In the 1950s, because of hundreds of accidental infections, and the deaths of three employees at Fort Detrick from biological warfare agents, (of which two deaths were due to anthrax) [8], a medical defense program was begun. Its purpose was to develop vaccines for the protection of laboratory workers engaged in research on biological warfare agents [9]. This program was successful, more stringent safety measures were applied, and the number of inadvertent infections of personnel at Fort Detrick decreased sharply [10].

In 1968, against the backdrop of mounting domestic and international opposition to the use of chemical weapons in Vietnam, a scandal surfaced in Skull Valley, Utah. Lethal nerve gases were accidentally sprayed 20 miles beyond the boundary of the Army's testing site at Dugway Proving Ground and killed 6,400 sheep. The Army refused to acknowledge its involvement in this affair for 15 months, until autopsy results and other data were so overwhelming that officials could no longer deny complicity. Then-Congressman Richard D. McCarthy, who had been a journalist in Buffalo, New York prior to entering Congress, wrote a book exposing this and a number of other problems with the U.S. chemical and biological warfare programs [11].

Perhaps as a way to deflect attention from these criticisms, President Nixon announced an important turn-around in policy on November 25, 1969, 1 day prior to publication of Congressman McCarthy's book [12]. Nixon stated,

> Biological weapons have massive, unpredictable, and potentially uncontrollable consequences. They may produce global epidemics and impair the health of future generations. I have therefore decided that: 1) The United States shall renounce the use of lethal biological agents and weapons and all other methods of biological warfare. 2) The United States will confine its biological research to defensive measures such as immunization and safety measures. 3) The Department of Defense has been asked to make recommendations as to the disposal of the existing stocks of bacteriological weapons [13].

The Nixon doctrine was codified by National Security Decisions 35 (issued on the same day) and 44 (issued on February 20, 1970), which stand today to define and limit the Army's biological defense mission [14,15]. The Nixon administration also led the United States to join with other nations in developing the Biological Weapons Convention (BWC), a treaty signed in 1972 by 108 nations and ratified by the U.S. Senate in 1975. Article I of the BWC states:

> Each State party to this convention undertakes never in any circumstances to develop, produce, stockpile, or otherwise acquire or retain:
>
> 1. Microbial or other biological agents, or toxins whatever their origin or method of

production, of types and in quantities that have no justification for prophylactic, protective, or other peaceful purposes;
2. Weapons, equipment or means of delivery designed to use such agents or toxins for hostile purposes or in armed conflict [16].

The terms of the treaty have been subjected to various interpretations over the years, however, and at the time it was written, no provisions were made for verifying compliance of signatory nations. After the Nixon declaration of 1969, for example, Henry Kissinger claimed that Nixon's order "does not preclude research into those offensive aspects of bacteriological or biological agents necessary to determine what defensive measures are required" [17]. This ambiguity has persisted, giving rise to the debate over whether the United States may produce offensive agents in order to test defenses against them. The line between offensive and defensive research, not strictly defined by Nixon in 1969 or the BWC in 1972, remains blurred.

THE CASE OF ANTHRAX

Anthrax, in nature, is a disease of animals that usually acquire the infection by grazing in fields where anthrax spores are present in the soil. An estimated 20,000 human cases per year, worldwide, occur as a result of contact with infected animals or animal products. Most of these human cases develop cutaneous anthrax, characterized by a necrotic ulcer and severe local swelling at the site of contact. The rarer syndrome of inhalation anthrax (previously known as woolsorter's disease) results from inhalation and pulmonary deposition of anthrax spores. Initially presenting as a flu-like illness, inhalation anthrax rapidly progresses to severe respiratory distress, after which death usually ensues within 24 hours. On clinical grounds, the disease is difficult to diagnose in its early stages. By the time the patient is severely ill, antibiotic treatment has little effect. The fatality rate is about 90%.

Anthrax was developed as a biological weapon during World War II because it has three useful qualities: 1) the production of a lethal toxin; 2) the production of a spore, which renders it impervious to extremes of temperature and pressure, allowing it to be sprayed from airplanes or exploded from bombs without losing virulence; and 3) the production of a capsule, which enables the organism to resist phagocytosis by the body's defenses, and thus to successfully invade and cause disease. In addition, there is essentially no human-to-human transmission, so a potential user need not fear uncontrollable spread of the disease back to his or her own territory. Disease is limited to the area of dissemination of the spores.

The first anthrax vaccine, an attenuated strain, was developed by Louis Pasteur in 1881, as anthrax was ravaging European livestock [18]. The current human vaccine was developed at Fort Detrick in the 1950s and 1960s and is a purified preparation of one anthrax protein, termed protective antigen. There are actually three toxin proteins: edema factor, lethal factor, and protective antigen. Edema factor, when injected, causes localized edema; and lethal factor causes death. Neither of these proteins can exert its toxic effect, however, without the presence of protective antigen, which serves as a receptor binding protein and allows these two toxin proteins to enter target cells. On the other hand, neither edema factor nor lethal factor are immunogenic. In other words, neither stimulates the production of antibodies that can protect against infection with anthrax. The third protein, protective antigen, has been conclusively shown to be the only immunogenic component of anthrax toxin [19].

The BDRP has suggested that the current human vaccine, a preparation of protective antigen that contains small admixtures of edema factor and lethal factor, is too reactogenic for widespread use, and therefore a better vaccine is needed [20]. To this end, protective antigen protein has been completely purified and crystallized [21]. In addition, Fort Detrick scientists have succeeded in transferring the protective antigen gene from Bacillus anthracis to the more benign Bacillus subtilis [19]. This genetically engineered bacterium produces protective antigen in even greater quantities than the original vaccine strain, making pure protective antigen easily obtainable. Yet despite achieving the capacity to produce pure protective antigen in large quantities, the need to produce protective antigen for a vaccine continues to be invoked by BDRP researchers as the justification for research in unrelated, more questionable areas [22].

BDRP-sponsored work on anthrax has resulted in publication of articles with titles such as "Molecular Cloning and Expression in Escherichia coli of the Lethal Factor Gene of Bacillus anthracis" [22], "Pu-

rification of Bacillus anthracis Lethal Factor by Immunosorbent Chromatography" [23], and "Production and Purification of Anthrax Toxin" [24]. This last paper describes production of toxin proteins in 50 liter batches. Other research was designed to learn the "maximum rate of killing" of various lethal factor and protective antigen ratios in rat lethality assays and time-to-death studies [25].

The study and production of toxin proteins (lethal factor and edema factor) may well have scientific merit. But such work raises questions regarding U.S. compliance with the BWC, as well as U.S. government doctrine expressed in the National Security Decisions of 1969 and 1970. A purely theoretical interest in the toxin might be pursued more appropriately within a civilian agency charged with supporting biomedical research. Transfer of control of this type of research from the Army to a civilian agency would dispel some of the concern about the offensive intent of the work. Nevertheless, it is hard to imagine why a nonmilitary researcher would be funded to investigate the optimal amounts of protective antigen and lethal factor needed to produce a lethal effect. On the other hand, if the Army were studying lethal factor in order to develop a treatment capability for anthrax, the research would be considered legitimate under the terms of the BWC and the National Security Decisions. This rationale for these experiments, however, has not been presented in any of the papers cited here, and since "all work conducted under the BDRP is unclassified" [26], there would be no reason to hide it.

Also disturbing is BDRP sponsored research on transfer of the plasmids of B anthracis [27]. Virulent strains of B. anthracis contain two plasmids, pX01 and pX02, both of which are required for virulence. pX01 encodes the three components of anthrax toxin, consisting of edema factor, lethal factor, and protective antigen, and pX02 encodes the synthesis of the capsule.

In research funded by the BDRP, genes specifying high-frequency cell-to-cell transfer of plasmids and coding resistance to erythromycin have been fused to the B. anthracis virulence plasmids; and an unrelated plasmid, carrying tetracycline resistance, has been introduced into B. anthracis. National Institutes of Health guidelines for recombinant DNA research prohibit the transfer of antibiotic resistance (by molecular cloning) to pathogenic species in which it does not occur naturally. Although these experiments did not involve cloning techniques, a similar restriction should apply.

In subsequent experiments, pX01 and pX02 of B. anthracis were physically joined to antibiotic resistance genes and transferred into Bacillus cereus. These composite plasmids, containing either the pX01 toxin genes or the pX02 capsule genes plus the erythromycin resistance gene, could be transferred to recipient cells at high frequency (1% per donor organism).

These experiments are disturbing because they involve the creation of previously unknown, antibiotic resistant Bacillus strains that are capable of transferring their resistance and virulence factors jointly and at high frequency. Bacterial strains capable of high-frequency transfer of antibiotic resistance and B. anthracis virulence factors offer the possibility of constructing a variety of novel organisms for use as bioweapons. Although interspecies transfer of these plasmids has been described thus far only between different Bacillus species, it is likely that transfer could be achieved with other bacterial species as well

The research described above is devoted solely to the study of the interbacterial transfer of the B. anthracis virulence plasmids, pX01 and pX02, and the genetics of this process. Yet the title of the BDRP grant under which the research was performed is "Genetic and Physiological Studies of Bacillus anthracis Related to Development of an Improved Vaccine" [28]. There is no published evidence to suggest that the research has addressed vaccine development. Even if one used an immunological justification for transferring pX01 to other bacteria, this justification would not apply to pX02, which lacks immunogenic properties. One must conclude that the research program described above has nothing to do with vaccine development; the potential for novel bioweapons outweighs any conceivable vaccine potential.

The BDRP produced an Environmental Impact Statement in April 1989. This document specifies the mission of the BDRP and discusses its role in relation to the research limits established by the National Security Decisions of the Nixon years and by the BWC. It lists three mission objectives, similar to Huxsoll's list: 1) development of biological agent detection methods, 2) development of treatment and protection capability, and 3) development of decontamination capability [26]. This delineation of the

BDRP's role would indicate that the study of toxins and development of new virulent strains, unless clearly linked to real vaccine enhancements or other forms of medical treatment, lie outside the permitted range of the BDRP's mission and activities.

STABILIZATION, DISSEMINATION AND DELIVERY OF BIOLOGICAL AGENTS

Production of a spore allows anthrax to be stabilized as a dried preparation, packaged into weapons, and disseminated as an aerosol. The absence of a spore prevents most disease-causing organisms from being developed for use in airborne weapon systems, since the dissemination process would destroy them. Production of a synthetic protective membrane, however, could circumvent this problem. Development of synthetic liposomes has been sought by biologists for a variety of nonmilitary medical and research indications, but the BDRP has taken an interest in it for microencapsulation as well [29].

Development of weapons delivery systems for biological warfare agents appeared to be the one area, identified by Huxsoll, for which there was no evidence of U.S. military involvement. That is, until 1987, when a lawsuit in federal court forced the release of a list of titles of all studies performed by the BDRP during the previous 5 years [30]. The content of the studies was not released. Yet the title of one, "Biological Agent Delivery by ICBM (Inter-Continental Ballistic Missile)," demonstrates that this final step toward weapons development in Huxsoll's list had not been completely ignored.

In fact, specific delivery systems probably do not need to be developed for biological warfare agents. In World War II, cluster bombs were filled with anthrax "bomblets" for delivery by airplane. The Japanese used hand grenades as one method of delivery. Other means of delivery mentioned in the published literature include guided missiles, airplane spray tanks, and a variety of bombs [31]. One could probably pick out a number of delivery systems from among those the military already has available [32]. And because delivery systems for chemical weapons are not yet prohibited by treaty, they are also available for easy adaptation to deliver biological warfare agents.

CONCLUSIONS

Evidence has been presented to demonstrate the broad scope and potential offensive applications of some of the BDRP's recent areas of research. The Department of Defense reported in 1980 the possibility that advances in recombinant DNA technology might enable "a potential enemy to implant virulence factors or toxin producing genetic information into common, easily transmitted bacteria such as Escherichia coli" [33]. This precise procedure using the anthrax lethal factor gene was performed for the BDRP in 1986 [22]. As with other weapons systems, fear of what an enemy might do feeds our own designs. The difference in this case is that research on biological warfare agents is strictly limited by United States policy and international law.

At best, the BDRP, in pursuit of defense, has transgressed the limits established by U.S. government policy, leading many here and abroad to be concerned about the ultimate effect on the Biological Weapons Convention. International perception of compliance by all the signatories is needed to maintain the power of this treaty, currently a model for international arms control.

At worst, the BDRP has deceived the public, ignored the law, and pursued the creation of new biological weapons.

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