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The intentional release of biological agents by belligerents or terrorists is a possibility that has recently attracted increased attention. Law enforcement agencies, military planners, public health offcials and clinicians are gaining an increasing awareness of this potential threat. From a military perpective, an important component of the protective pre-exposure armamentarium against this threat is immunization. In addition, certain vaccines are an accepted component of postexposure prophylaxis against potential bioterrorist threat agents. These vaccines might, therefore, be used to respond to a terrorist attack against civilians. We review the development of vaccines against 10 of the most credible biological threats.

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Immunization against Potential Biological Warfare Agents

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The intentional release of biological agents by belligerents or terrorists is a possibility that has recently attracted increased attention. Law enforcement agencies, military planners, public health officials, and clinicians are gaining an increasing awareness of this potential threat. From a military perspective, an important component of the protective pre-exposure armamentarium against this threat is immunization. In addition, certain vaccines are an accepted component of postexposure prophylaxis against potential bioterrorist threat agents. These vaccines might, therefore, be used to respond to a terrorist attack against civilians. We review the development of vaccines against 10 of the most credible biological threats.

The possible use of biological agents as weapons of warfare or vehicles for terrorism has generated considerable recent interest in both the lay [1, 2] and scientific [3–6] press. Public awareness of the threat posed by biological agents adapted for sinister purposes has been highlighted by movies such as *Outbreak,* by popular books such as *The Cobra Event* and *The Eleventh Plague,* and by myriad press accounts of the activities of groups such as the Aum Shinrikyo cult in Japan. The latter, for example, gained notoriety by releasing nerve agent in the Tokyo subway system but also possessed and experimented with anthrax spores and botulinum toxin [7].

With this increasing awareness has come a growing attempt to defend against the possibility of biological warfare and terrorism. Military units and civilian law-enforcement agencies have begun to train crisis-response teams to prepare for biological contingencies. The possibility of an attack with biological agents is now often included in "war-gaming" exercises and counter-terrorism planning that are conducted by agencies such as the Department of Defense (DoD), Centers for Disease Control and Prevention (CDC), Federal Emergency Management Agency (FEMA), Federal Bureau of Investigation (FBI), and others. Despite these efforts and our best diplomatic measures, however, the risk that biological agents will be used in warfare or terrorism appears to remain quite high. Reasons for this include the relatively low degree of technological sophistication and expense required to produce a biological weapon compared to those of other weapons of mass destruction, such as chemical and nuclear arms. With this in mind, it seems improbable that increased awareness, sophisticated surveillance, and rapid crisis response will fully prevent all attempts at biological aggression. Therefore, one of the best defenses, especially in a military context, will probably continue to be vaccines, and this requires the development of new and improved vaccines and treatments against the relatively small handful of viable biological warfare agents. Although civilian planners are unlikely, in the near future, to employ such vaccines prospectively, they may, in some cases, consider vaccination in a consequence management context following a biological terrorist assault on civilians. For example, both anthrax and smallpox (vaccinia) vaccines are accepted components of postexposure prophylaxis for these diseases.

Biological warfare agents may be classified in several ways: (1) operationally, as lethal or incapacitating agents, and as agents with or without potential for secondary transmission; (2) according to intended target, as antipersonnel, antianimal, antiplant, or antimateriel; and (3) according to type, as replicating pathogens, toxins, or biomodulators. In this review, we consider 10 antipersonnel agents that are the most credible warfare and terrorism threats. Included among these agents are both replicating pathogens (bacteria and viruses) and toxins.

Bacterial Diseases

Anthrax. One of the few encouraging aspects of the daunting task of biowarfare defense is that few agents possess characteristics suitable for effective large-scale employment. No agent, however, has properties as ideal as those of *Bacillus anthracis.* Its ubiquitous presence in soil and the simplicity of culturing it make anthrax readily available to armies and to terrorists. And its lethality, its ability to form resilient spores, and its capacity for aerosolization combine to make anthrax one of our greatest biological threats. Anthrax was preeminent in the arsenals of Iraq and the former Soviet Union; the Aum

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Table 1. Various licensed and investigational new drug (IND) vaccines potentially useful against biological-warfare agents.

Agent	Vaccine name	Antigen	Year(s) developed	Regimen	Status and availability	References
Anthrax	Live Spore Vaccine	Whole spore, attenuated	1937	10–20 μL id (scarification)	Used in some countries; similar to veterinary vaccines	[10]
	Anthrax Vaccine, Adsorbed	PA	1960s	0.5 mL sc at weeks 0, 2, and 4, and at months 6, 12, and 18; annual boosters	FDA-licensed, 1970	[9, 20]
	United Kingdom Vaccine	PA, LF	1950s	0.5 mL sc at weeks 0, 3 ,6, and 32; annual boosters	Used in Great Britain	[9]
Plague	Plague Vaccine, USP	Whole-cell, formalin-killed	1942	1.0 mL im, then 0.2 mL im at months 1, 3, 4, 5, and 6; boosters q 6 mo × 2, then annually	FDA-licensed; currently not in production	[24, 28]
Tularemia	Live Vaccine Strain	Live, attenuated	1956	0.1 ml pc[c] (scarification)	IND[a]	[45]
Q-Fever	Q-Fever Vaccine	Whole-cell, formalin-killed	1937	0.5 mL sc	IND;[a] licensed in Australia as Q-Vax	[51]
	CMR	Whole-cell extract	1990s	0.5 mL sc	IND	[53]
Smallpox	Dryvax	Live vaccinia virus	1960s	~2.5 μL pc (scarification); boosters q 3–5 y	FDA-licensed; not in production[b]	[55]
	Cell-Culture–Derived Vaccine	Live vaccinia virus	1990s	~ 2.5 μL pc (scarification)	IND	[56]
VEE	TC-83	Live, attenuated	1961	0.5 mL sc	IND[a]	[57, 59]
	C-84	Formalin-inactivated	1970s	0.5 mL sc to TC-83 non-responders	IND[a]	[59, 60]
Botulism	Botulinum Toxoid, Adsorbed	Pentavalent toxoid (types A, B, C, D, E)	1950s	0.5 mL sc at weeks 0, 2, and 12; annual boosters	IND[c]	[66]

NOTE. CMR, chloroform-methanol residue; id, intradermally; LF, lethal factor; PA, protective antigen; pc, percutaneously; USP, United States Pharmacopaeia.
[a] These permits are held by the Joint Program Office for Biological Defense (JPO-BD), an agency of the US Department of Defense.
[b] The Centers for Disease Control and Prevention (CDC) maintains supply of vaccine for those at risk of occupational exposure to orthopoxviruses.
[c] These IND permits are held by the CDC or the JPO-BD.

Shinrikyo cult also stockpiled it. The World Health Organization (WHO) [8] estimates that the release of 50 kg of anthrax spores along a 2-km line upwind of a city of 500,000 people would produce 125,000 infections and 95,000 deaths, far more than with any other agent considered. Not surprisingly, research programs at military laboratories have devoted considerable effort to improving on the anthrax vaccines that have been in use for many decades.

Anthrax vaccination dates from 1881, when Pasteur's veterinary preparation became the first bacterial vaccine adopted for general use in cattle and sheep, just 5 years after anthrax became the first disease for which a microbial etiology was proven by Koch's postulates. Pasteur's duplex vaccine, which consisted of 2 doses prepared by significantly different methods, remained in wide usage in Europe and South America until it was modified in the 1930s and eventually gave way to Sterne's live spore vaccine in 1937. Derivatives of this attenuated spore vaccine are used extensively in livestock to this day and are remarkably successful in controlling anthrax in many areas of the world. A thorough review of the history of early anthrax vaccines has been published [9].

Because of significant vaccine-associated morbidity, the use of live spore preparations is problematic in humans. Nonetheless, a live spore vaccine derived from a Sterne strain of *B. anthracis* is still used in humans in the former Soviet Union [10]. A new generation of vaccines with more acceptable side-effect profiles were developed after the 1950s and 1960s, when the 3 principal protein virulence factors in *B. anthracis* were delineated, namely, protective antigen (PA), lethal factor (LF), and edema factor (EF) [11, 12]. Pathogenesis of tissue edema and necrosis seen in anthrax cases is a result of the effects of LF and/or EF, which form the "A" chain of anthrax toxins according to the A-B model of dichain toxins [13]. PA serves as the "B" chain transport protein, which binds to receptors on target cell membranes and initiates toxin uptake. Since the appreciation of the role of PA in immunity, vaccines prepared in the United Kingdom in the 1950s and in the United States in the 1960s have employed cell-free filtrates that optimize the content of PA. Because of the success of veterinary vaccines and the very low incidence of human anthrax in the Western world, to date there has been little impetus for improving these vaccines, which remain in use today. The vaccine currently available for human use in the United States was licensed in 1970, and is derived from strain V770, a PA-rich organism originating from a case of bovine disease. A vaccine in use in Britain derives from the Sterne strain, but is prepared in a somewhat similar manner [9]. Table 1 presents basic information comparing the United States and United Kingdom vaccines, as well as other licensed and investigational "anti-BW" vaccines.

During the past several years, interest in the development of new anthrax vaccines has increased. Such interest has been prompted by a significant (defined as an inflammatory reaction >5 cm in diameter) local reaction rate of 2.4%–3.9% associated with the current vaccine (unpublished data, US Army Medical Research Institute of Infectious Disease [USAMRIID]), persist-

ence for several weeks of subcutaneous nodules at injection sites, and an unwieldy immunization schedule. Moreover, awareness of the anthrax threat has increased as a result of the Sverdlovsk incident [14], the discovery of the Iraqi and Aum Shinrikyo arsenals, and myriad hoaxes perpetrated throughout the United States. Finally, implementation of the DoD anthrax vaccination program throughout the armed forces has dramatically increased the size of the vaccinee population, amplifying the number of minor vaccine reactions reported.

At least 3 basic approaches have been employed in the search for a new anthrax vaccine [15]: (1) recombinant vaccines, in which the PA gene is cloned into organisms of low pathogenicity such as *Bacillus subtilis;* (2) mutant-strain vaccines derived from the Sterne strain but dependent on aromatic compounds not found in human tissue; and (3) purified PA preparations combined with various adjuvants. All 3 approaches have been studied by researchers at the USAMRIID and have led to the development of experimental vaccines that protect against aerosolized spore challenge in guinea pigs [16–18]. In particular, a vaccine candidate composed of purified PA combined with monophosphoryl lipid A appears promising. In guinea pigs, this vaccine proved superior in efficacy to the currently available human vaccine [18]. Moreover, lyophilization appeared to have no effect on vaccine potency—a potential advantage over the current preparation, which requires a cold storage chain. Finally, a DNA plasmid vaccine, which incorporates the immunogenic and biologically active portion of PA has demonstrated protection in mice against lethal challenge with a preparation of PA and LF [19].

In addition to developing these candidate vaccines, much of the effort currently taking place at USAMRIID involves attempts to reduce the number of doses in the licensed vaccine regimen without reducing vaccine efficacy. The current anthrax vaccine is given as a 6-dose series of 0.5-mL sc injections at 0, 2, and 4 weeks; and at 6, 12, and 18 months. Boosters are administered annually for persons at continuing risk of exposure. The BioPort Corporation (Lansing, MI), under contract to the DoD, produces the vaccine, which may also be useful after exposure. In the event of an anthrax release, because of the possibility of delayed germination of spores within the body, anthrax vaccine has been recommended as an adjunct to postexposure antibiotic prophylaxis [20].

Plague. One of the earliest recorded attempts at biological warfare was the effort of besieging Tatar warriors to catapult the corpses of their own plague victims over the city walls of Kaffa in the Crimea in order to initiate an epidemic within the city [21]. The Japanese studied plague extensively as a potential biological weapon before and during World War II. In their "experiments," millions of infected fleas were released over Manchurian cities, resulting in numerous human plague cases [22].

Plague vaccination dates to Haffkine's use of a killed preparation (the "plague prophylactic fluid") in India in 1897 [23]. Although apparently effective, occasional cases of breakthrough disease, combined with an inordinately high rate of adverse reactions, caused vaccine recipients and the scientific community to reject the preparation [24]. The high rate of adverse reactions stemmed from the erroneous belief that doses sufficient to cause systemic febrile reactions were necessary to induce protective immunity. Before World War II, a new-generation, formalin-killed modification of the original Haffkine vaccine was prepared from *Yersinia pestis* strain 195/P, a virulent clinical isolate from India. On the basis of safety studies, the National Research Council Committee on Medical Research recommended the use of such killed vaccines in military and naval personnel. Approximately 12,000 troops received the 2-dose vaccine series (with Plague Vaccine, United States Pharmacopaeia, an Army version of the formalin-killed preparation) during World War II; none contracted plague, despite their deployment in areas of high endemicity [24].

During the Vietnam War, plague vaccine was routinely administered to members of the US armed services, and only 8 cases of plague were reported among this population [25], which corresponds to a rate of ~1 case per million person-years of exposure. The success of this vaccine is evident when compared with the 330-fold greater incidence of plague among the unvaccinated South Vietnamese civilian population [26], and when compared with the relatively high incidence among US troops of murine typhus, another disease transmitted in Vietnam by the same vector, *Xenopsylla cheopis* [27].

Despite the success of plague vaccine in protecting soldiers against endemic plague, this preparation may not protect adequately against acquisition of disease by the aerosol route [28, 29], which is the likely avenue of exposure in the event of a biological attack. In addition, a large proportion of vaccine recipients report local reactions to plague vaccine, and reaction rates increase with successive doses. Hence, it is recommended that after a first dose of 1 mL, the inocula be reduced to 0.2 mL for the second and third immunizations (at 1–3 months and 5–6 months, respectively) of the 3-dose primary series [28]. In addition, 20% of vaccine recipients report systemic reactions to plague vaccine [30]. Seven percent of vaccine recipients fail to respond serologically, whereas many others require the full 3-dose series to achieve hemagglutination titers similar to those protective in mice [31]. Finally, the short duration of immunity elicited by this vaccine necessitates booster doses as often as every 6 months [28]. These difficulties, as well as supply and manufacturing problems, highlight the need for improved vaccines effective against *Y. pestis,* and particularly against plague transmitted by aerosol.

One theory guiding the search for an improved vaccine is that mucosal immunity in the tracheobronchial tree may be important in the defense against pneumonic plague [32]. Oral immunization of vervets with a live-attenuated *Y. pestis* vaccine, EV76, afforded some protection against inhalational challenge [33]. However, EV76 and similar live plague vaccines produced

a significant number of side effects when administered subcutaneously to humans [24]. Another avenue of investigation involves the use of recombinant F1 antigen as an immunogen. Although antibody against this peptide protects against plague, F1 is not critical for virulence, and virulent F1-negative *Y. pestis* strains are known [34]. More recently, subunit vaccines containing multiple antigens (namely, F1 and V) have been shown to protect mice against pneumonic disease [35, 36].

Brucellosis. The causative agents of brucellosis are categorized as incapacitating agents, with infections by aerosol likely to produce large numbers of casualties but little mortality. Nevertheless, brucellosis deserves consideration by defense planners because of its extraordinary infectivity. In fact, in its era of offensive biological warfare research in the 1950s, the United States chose *Brucella suis* as the first agent to be produced at the newly constructed Pine Bluff Arsenal in Arkansas [37].

Veterinary vaccines that have significant efficacy against brucellosis have been studied and employed. The vaccination of livestock to reduce enzootic disease load, in combination with the slaughter of infected animals, is largely responsible for the declining incidence of human brucellosis. In the United States, the decline of human brucellosis cases reported to the CDC has paralleled the control of infections due to *Brucella abortus* in cattle [38]. Most veterinary vaccines in use today derive from *B. abortus* strain 19, an attenuated organism with stable virulence, or from Rev 1, a live, virulence-stable *Brucella melitensis* strain. A review of the role of brucellosis vaccination in veterinary medicine has been published [39].

No licensed human vaccine against brucellosis is available in most of the Western world, including the United States, although live *Brucella* vaccines have been employed at various times in many developing nations [40]. Most preparations were derived from *B. abortus* strain 19, reflecting the cross-immunity among *Brucella* species and diminished human virulence of *B. abortus* when compared with other species. Nonetheless, administration of either live preparation to humans is hampered by a modest but notable incidence of clinical brucellosis cases, as well as by significant hypersensitivity reactions. Such problems were noted in the former Soviet Union, where human vaccination is still widely employed, and in a US trial of strain 19 and Rev 1 vaccines conducted 35 years ago [41].

There have been several attempts to develop fractional component vaccines derived from various *Brucella* strains. An acetic-acid extract of a variant of *B. abortus* strain 19 that yielded a complex known as "brucellosis protective antigen" was tested in the USSR [42]; immunity elicited in guinea pigs failed to persist. A "phenol-insoluble fraction" vaccine, consisting primarily of delipidated strain 19 components, protected mice and guinea pigs against challenge, and has been used on a small scale in humans [43]. Although apparently effective in protecting high-risk vaccine recipients, reimmunization appears necessary every 2 years. Vaccination is further complicated by the need for skin testing before reimmunization, since skin-test–positive patients react strongly to vaccine [43]. More recently, outer membrane protein (OMP) of *Neisseria meningitidis* has been shown a useful adjuvant to intranasal *B. melitensis* immunization in mice and guinea pigs [44]. A mucosal vaccine combining OMP with purified lipopolysaccharide of *B. melitensis* is under study.

Tularemia. *Francisella tularensis* is sometimes considered a lethal biological warfare agent, since high-dose aerosol dissemination would result in a disproportionate number of cases of the pneumonic form of tularemia. *F. tularensis* followed *B. suis* into weapons production at Pine Bluff in 1955 [37], and extensive testing of the weaponization potential of the agent was conducted in human volunteers at Fort Detrick in a 1-million-liter sphere (the "eight ball") designed for such purposes. Moreover, the organism is believed to have been prominent in the biological arsenal of the Soviet Union. Ironically, the current live investigational vaccine is the result of an unprecedented cooperative effort during the height of the Cold War: the original vaccine strain was obtained in 1956 from the USSR's Gamaleya Institute. It derives from "strain 15," biotype *palearctica,* an organism attenuated by repeated subculture. In fact, descendants of this strain, commonly referred to as "live vaccine strain" (LVS), provide the seed stock for tularemia vaccines in use throughout the world.

LVS immunization has several limitations, as recently reviewed [45]. Among these is an incomplete knowledge of factors responsible for virulence in *F. tularensis* and of factors related to the genetic stability of the vaccine strain. Concerns remain that the vaccine strain could revert to a more virulent state or, alternatively, a less protective one, as has occurred among strains of *F. tularensis* vaccine previously employed in the Soviet Union [45]. Moreover, the vaccine strain presents 2 phenotypes, only 1 of which appears immunogenic. These factors dictate that each new lot of tularemia vaccine be evaluated for immunogenicity [46]. The search for improved vaccines is driven by the requirements for this cumbersome testing, for administration by scarification (a one-time 0.1-mL dose is applied to the skin and inoculation is accomplished with 16 stabs of a bifurcated needle), as well as by the ill-defined nature of the vaccine strain and the incidence of cases of tularemia among vaccine recipients receiving larger inocula. This search is hampered by uncertainty about the nature of the antigens required for protective cell-mediated immunity and by the failure of killed vaccines to produce such immunity [45]. LVS vaccine, as well as several other vaccines mentioned in this article (table 1), is available on investigational new drug (IND) protocol through the DoD's Joint Program Office for Biological Defense (JPO-BD) in Falls Church, Virginia.

Q fever. *Coxiella burnetii,* the causative agent of Q fever, is a pleomorphic gram-negative coccobacillus resistant to heat and desiccation that grows easily to high titer in embryonated chicken eggs and is highly infectious by aerosol. These factors explain the consideration given this organism as a potential incapacitating agent; *C. burnetii* was another of the 10 biolog-

ical weapons in the US arsenal destroyed after the renunciation of offensive biological warfare in 1969 [47].

The Smadel vaccine [48] was a highly immunogenic, formalin-inactivated preparation made from the Henzerling strain of *C. burnetii.* The history of this and related early vaccines has been reviewed [49]. Although apparently effective, early vaccines occasionally produced severe complications, including the formation of sterile abscesses and sinuses at the inoculation sites [50]. Improved Q fever vaccines followed the discovery of the phenomenon of phase variation among *C. burnetii.* The organism is pathogenic and immunogenic in its phase I form, but reverts to its avirulent, nonimmunogenic phase II form after serial passage in yolk sac cultures. This phase shift permitted the manufacture of vaccines formulated entirely of phase I organisms, with consequent improvement in immunogenicity.

The current Q fever vaccine, available under IND protocol through JPO-BD, is a formalin-inactivated, purified, Henzerling strain, phase I whole-cell preparation administered in a single 0.5-mL sc dose. A similar vaccine is licensed in Australia as Q-Vax (Commonwealth Serum Laboratories, Melbourne, Australia), and is highly effective at preventing clinical Q fever in humans [51]. Concerns remain, however, about its potential to produce severe local reactions in patients with immunity to *C. burnetii.* Use of a skin test, consisting of 0.1 mL of vaccine (administered intradermally), to identify these patients can reduce the incidence of local reactions but adds to the cost and complexity of immunization efforts. This need for skin testing might be eliminated with the development of the chloroform-methanol residue (CMR) vaccine. The CMR vaccine contains the phase I antigens and, in animal studies, is less reactogenic than Q-Vax, with similar efficacy [52]. In humans, CMR vaccine was safe and immunogenic in nonimmune volunteers [53]; it is currently being examined at USAMRIID for reactogenicity in individuals immune to Q fever.

Viral Diseases

Smallpox. Although endemic smallpox was eradicated throughout the world in 1977, the virus remains a potential biological weapon in the eyes of many military planners. Concerns persist that clandestine stocks of virus may exist outside of CDC in Atlanta, Georgia, and Koltsovo in Russia, the 2 WHO-authorized repositories of the virus. Moreover, the possibility exists that other orthopoxviruses might be genetically manipulated to produce virulent organisms similar to variola.

Over the years, the numerous, often poorly characterized, strains of vaccinia virus previously used in vaccination were abandoned at the recommendation of the WHO. Ultimately, derivatives of 3 strains remained available: the Lister-Elstree strain, the EM63 strain (used in the former Soviet Union), and the New York City Board of Health (NYCBOH) strain, an isolate of low pathogenicity [54] used in vaccine production by Wyeth Laboratories. A fourth strain, the "Temple of Heaven" strain, remained in widespread use in the People's Republic of China. The Wyeth product, Dryvax, is currently available through the CDC. It is administered by scarification with a bifurcated needle dipped in vaccine; further guidelines for its use are published [55]. The effectiveness of vaccinia vaccine is attested to by its success in smallpox eradication. A major problem exists, however, with future availability. Wyeth ceased manufacture of Dryvax in 1982; the CDC holds the remaining vaccine stocks, roughly 12 million doses, but these lots are gradually losing potency and will all ultimately expire. Potential manufacturers have little interest in resuming vaccinia production because of lack of economic incentives, concern over medicolegal risks, loss of production plant infrastructure, and uneasiness regarding use of a vaccine produced in calf lymph and administered in such an imprecise manner as scarification.

In light of these problems, investigators at USAMRIID have developed a new vaccine at the request of the DoD. This cell-cultured preparation derives from a Connaught (NYCBOH) vaccine strain in use until the early 1970s and recently underwent phase II testing in human volunteers. In this study, subjects who received cell culture vaccine intradermally and subsequently developed cutaneous pox lesions had immune responses similar to subjects who received Dryvax by scarification. Those recipients who failed to develop pox lesions or who received vaccine intramuscularly had humoral immune responses inferior to those elicited in the Dryvax group [56]. Although we anticipate that the cell culture vaccine will prove to have immunogenicity similar to the currently licensed product, it is also clear that administration by scarification remains necessary. An expanded trial is ongoing that compares Dryvax and the cell-culture-derived preparation, both administered by scarification.

Venezuelan equine encephalitis (VEE). Early attempts at immunization against VEE, an incapacitating agent, culminated in 1961 the development of a live-attenuated vaccine, TC-83 (representing the 83d passage in cell culture) [57, 58]. Although apparently effective, given the subsequent marked decline in laboratory-acquired VEE infections, TC-83 vaccination is complicated by a high systemic reaction rate [57] and an 18% rate of serologic nonresponders [59]. Although a formalin-inactivated preparation, C-84, is less reactogenic [60], it failed to protect against aerosol challenge in hamsters [61], which relegated it to its present role as a booster immunization for those with insufficient or waning titers after receiving TC-83 [59]. TC-83 and C-84 vaccines are available under IND protocol through JPO-BD. Current employment strategy for these vaccines involves administration of a 0.5-mL sc dose of TC-83, followed 28 days later by an assessment of plaque-reduction neutralization titers. Recipients with titers <1 : 20 are then given a 0.5-mL sc dose of C-84. Subsequent titer checks are performed annually on recipients at ongoing risk, with C-84 boosters given when titers fall below 1 : 20.

A recombinant attenuated VEE vaccine is in development.

This vaccine, V3526, has mutations at 2 loci that minimize the risk of reversion to virulence and may cause it to be less reactogenic than TC-83. V3526 protects mice against intranasal challenge with fully virulent VEE virus [62].

VEE virus is also being studied as a potential vector for delivery of other recombinant vaccines. These vaccine vectors, or replicons, are developed by substituting genes that code for a protein of interest (e.g., an immunizing epitope of a different virus or bacteria) for those that code for VEE structural proteins. The result is a viral genome that encodes its own replicases and transcriptases, enabling the synthesis of abundant quantities of mRNA coding for the protein of interest. The replicon genomes can be encapsidated into virus-like particles by cotransfecting replicon RNA along with helper RNAs that code the VEE nucleocapsid and capsid proteins. These virus-like particles contain the recombinant RNA genome. Following inoculation, the particles are taken up by immune effector cells; heterologous antigens are expressed, and protective immunity results. Since the replicon genome lacks the genes for VEE structural proteins, no viral progeny are produced. Consequently, the infection is limited to one cycle; viremia does not develop, and immunity to VEE structural proteins does not result [63]. This approach has been successful in immunizing rodents against Ebola and Marburg viruses, and non-human primates against Marburg virus [64]. Replicons could theoretically be developed to code for multiple antigens, conferring immunity against numerous pathogens. Since immunity to VEE structural proteins does not result, VEE replicons could theoretically be used repeatedly for booster immunizations, or for sequential immunizations against numerous pathogens, without being inactivated by host immunity. This is a potential advantage over vaccinia- and adenovirus-vectored recombinant vaccines [65].

Toxin-Mediated Diseases

Botulism. Iraq chose to weaponize botulinum toxin during the Gulf War in 1991, although its usefulness as a weapon might be limited by its instability during storage and modest range upon aerosolization. Nonetheless, when delivered by aerosolization, botulinum toxins would be expected to produce cases of typical clinical botulism. Moreover, terrorists might also use botulinum toxins to sabotage food supplies.

The low incidence of naturally occurring botulism has hindered vaccine development, and no licensed product exists today. An investigational pentavalent (types A, B, C, D, and E) toxoid, prepared by combining separate aliquots of the 5 inactivated toxins, is produced by BioPort under contract to the US Army. This preparation is little changed from an original Parke-Davis version [66], save for a decrease in the amount of residual formaldehyde to ameliorate the high rate of local reactions. It has been administered to several thousand volunteers and at-risk laboratory workers as a 3-dose series (0.5-mL sc doses at 0, 2, and 12 weeks) with annual boosters. Immunization is hampered by an increasing rate of local reactions to each subsequent booster. These local reactions, coupled with cold-storage requirements, less than desirable antibody titers to types B and D toxin [66], omission of types F and G toxoids, and high cost, have spurred efforts to develop improved botulinum toxoids. Recently, USAMRIID investigators [67] used recombinant technology to express a fragment of the heavy chain of botulinum toxin serotype A in *Escherichia coli,* which protected mice from intraperitoneal challenge with type A botulinum toxin.

Staphylococcal enterotoxin B (SEB) intoxication. SEB is one of several pyrogenic exotoxins produced by *Staphylococcus aureus,* and is considered a viable incapacitating agent by many planners. Although many clinicians are familiar with SEB as a cause of food-borne disease, its use in biological warfare might well involve aerosolization, whereby it would cause a systemic febrile illness accompanied by pulmonary symptoms. In a recent report [68], a laboratory accident caused 9 workers to develop inhalational SEB disease. Fever was prominent in all 9, with temperatures reaching as high as 41°C. All had cough, in most cases accompanied by dyspnea and chest pain.

No SEB vaccine is currently available for use in humans, although several approaches to immunization against SEB intoxication have been explored. Formaldehyde-inactivated SEB toxoid, prepared as an alum precipitate, has been incorporated into microspheres and proteosomes. Microspheres containing SEB toxoid that are administered im with an intratracheal booster have been shown to protect rhesus monkeys against aerosol challenge [69]. Another promising approach involves induced mutations in the SEB protein which render the molecule nontoxic yet leave its three-dimensional antigenic structure intact. Using an SEB superantigen preparation with 3-point mutations, investigators demonstrated immunogenicity in rhesus monkeys without apparent toxicity [70]. We anticipate that such a preparation will provide protection against aerosol challenge.

Ricin intoxication. Ricin, a glycoprotein derived from the castor bean, is extremely toxic by the oral, respiratory, and percutaneous routes, and has been used in multiple assassination attempts [71]. Its ease of extraction from the waste mash of castor oil production, and the worldwide availability of castor beans, makes ricin a putative lethal agent of biological warfare and terrorism.

There is no effective human vaccine against ricin intoxication. A formalin-inactivated toxoid [72] and a deglycosylated A-chain subunit vaccine (unpublished data, USAMRIID) both protect mice against aerosol challenge, and encapsulation of toxoid in microparticles protected mice after a single vaccine inoculation [73]. Greater safety and less reactogenicity may make the subunit vaccine a more likely candidate for regulatory approval.

Summary

The DoD tasks USAMRIID with "conducting research to develop strategies, products, information, procedures, and training for medical defense against biological warfare agents." Vaccines are a critical component of these defense strategies as they apply to uniformed military personnel. Licensed vaccines exist against anthrax, smallpox, and plague. In addition, IND products are administered at USAMRIID to protect at-risk laboratory personnel against tularemia, Q fever, VEE, and botulism, and against diseases such as Eastern and Western Equine Encephalitis, Rift Valley Fever, and others. Recently, the DoD has embarked upon an anthrax immunization campaign throughout the armed forces, and it is quite conceivable that other anti–biological warfare vaccines will eventually be employed to protect soldiers, sailors, airmen, and marines. Finally, vaccines against other biological agents, as well as improved vaccines against agents listed above, are in various stages of research and development. In a civilian context, use of these vaccines is more problematic, because the nature of the threat is less well defined. Nonetheless, certain vaccines, such as anthrax and smallpox, may have applicability in the postexposure prophylaxis and management of exposed civilian populations.

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