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New Emerging Infections: Their Development, Testing and Resulting Diseases

Garth L. Nicolson, Ph.D. and Nancy L. Nicolson, Ph.D.

The Institute for Molecular Medicine, Huntington Beach, California 92647

Hypothesis: The emergence of new diseases and the increase in incidence rates of those with previously described signs and symptoms from the 1970s onward are due to our increasingly toxic environment, the emergence of previously isolated infections and the purposeful development and testing of new Biological Weapons of Mass Destruction agents.

Introduction

Over the last 70 years a tremendous worldwide effort has been undertaken to develop new Biological Weapons of Mass Destruction (BWMD) agents. Requirements for these new agents are that they must be colorless, by-and-large odorless microorganisms (bacteria, viruses, fungi) or toxins derived from these microorganisms that can be spread in air as aerosols or in food or drink or by biting insects to infect as many people as possible.[1] They must be easy to conceal, and thus difficult to detect before an attack. They must also be difficult to detect when released, so a biowarfare or bioterrorist attack would be difficult to ascertain, especially due to the usually nondescript initial signs and symptoms expected in casualties from an attack. Another advantage is that they allow the perpetrators easy avoidance or escape while eventually causing panic and chaos within a civilian or military population while overwhelming emergency medical departments at local hospitals and clinics or military medical facilities.

A misconception about BWMD agents is that they would have to cause large numbers of immediate deaths to be effective. Most BWMD agents do not cause widespread immediate deaths, or even large numbers of deaths within days of exposure, and most exposed patients might not even have a life-threatening disease in order to cause panic and terror. The main function of BWMD is to cause disruption and chaos, so BWMD agents don't have to cause a fatal disease to be effective. In fact, many biological warfare agents are categorized as 'incapacitating agents' that are not intended to produce fatal disease. They are more effective if they incapacitate and produce strain on a health care system and economic infrastructure by having many thousands of sick patients inundate treatment facilities that contain only limited quantities of drugs and only a few isolation wards.

It is much easier to spread a BWMD incapacitating agent from person to person, because it would not cause enough alarm to require quarantining of exposed persons, thus causing a failure

to limit additional exposures. BWMD incapacitating agents often have relatively long incubation times, allowing their widespread penetration into a population before they are ever diagnosed or detected by laboratory tests. Thus exposed individuals may bring the agent back 'home' to an unsuspecting family. This may have happened to veterans who returned from the first Gulf War only to slowly spread their chronic illnesses to spouses and children.[2]

Biological Agents Used for BWMD

There are several biological agents that could be useful as BWMD. First, there are lethal agents, such as the Ebola, Lassa and other viruses that cause viral hemorrhagic fever, inhalation anthrax caused by *Bacillus anthracis*, small pox virus, pneumonic plague caused by *Yersinia pestis* or purified protein toxins, such as the *Ricinus communis* toxin ricin or *Clostridium botulinum* toxin (Table 1). In addition, there are incapacitating agents, such as brucellosis caused by *Brucella* species, Q fever caused by *Coxiella burnetii*, tularemia caused by *Francisella tularensis*, mycoplasmal infections caused by *Mycoplasma fermentans* and mold toxins, such as the T2 mycotoxin. As mentioned above, the incapacitating agents for the most part cause chronic illnesses that are not usually fatal. However, these illnesses can cause tremendous health problems in infected patients, and some are contagious and the disease could spread.

Table 1. Some Common Biological Warfare Agents Useful as BWMD

Disease or agent	*Lethality (death)*	*Incubation period*	*Effective dose*	*Environmental stability*
Lethal Agents				
Anthrax	high	1-6 d	8,000-50,000 spores	very stable for years
Plague	high	1-6 d	100-500 organisms	stable for 1 year
Smallpox	high	7-17 d	10-100 organisms	very stable
Ebola virus	high	2-6 d	10-100 organisms	unstable
Botulism	high	1-5 d	0.001 mcg/Kg weight	relatively stable
Ricin	high	1-2 d	3-5 mcg/Kg weight	stable
Cholera	high	1-3 d	10-500 organisms	unstable
Incapacitating Agents				
Brucellosis	low	months	10-100 organisms	very stable
Tularemia	low	2-15 d	10-50 organisms	stable for months
Q fever	low	15-40 d	1-10 organisms	stable for months

Mycoplasma	low	months	10-100 organisms	moderately stable
T-2 Mycotoxins	moderate	1 d	unknown	very stable
Type B Enterotoxin	moderate	<1 d	0.03 mcg	moderately stable
Equine encephalitis	low	2-6 d	10-100 organisms	relatively unstable

Many of the most lethal BWMD, such as the hemorrhagic fever viruses, are quite unstable in the environment due to their susceptibility to sunlight and temperature and would not be effective if deployed in an aerosol at long range by aircraft or missile. However, others, such as anthrax spores, can easily survive using this method of dispersal. Anthrax spores have been found to survive for over 50 years in the environment, even under extreme conditions. Although anthrax spores are considered one of the most likely biological agents that might be deployed, once the illness occurs it cannot be commonly spread to others, unlike many of the incapacitating agents. Other highly lethal infections, such as from hemorrhagic fever viruses, show unique signs and symptoms that would allow isolation of the victims and thus prevent further spread of the disease. Many of the bacterial or viral incapacitating agents, however, could slowly produce illnesses that would not be noticeable until some time later, and during this period they could be slowly spread to others.

Development of New BWMD Agents

Over the years new BWMD agents have been developed, but their testing has remained difficult due to the fact that most nations involved in BWMD development are signatory to the 1972 Convention on the Prohibition of the Development, Production and Stockpiling of Biological Weapons. For example, the USA ratified this international treaty in 1975. This treaty bans the development and testing of all BWMD agents. However, signatory nations have circumvented this ban by conducting secret experiments under the guise of biodefense research, which is permitted under the treaty, or by flagrant disregard of the terms of the treaty.

The authors' inadvertent involvement in discovering the unethical program began when military casualties returned home from Gulf War I with undiagnosed chronic illnesses that could not easily be assigned to the normal military diagnosis categories. Eventually this became known as Gulf War Syndrome or Gulf War Illness.[2] Over the years tremendous effort has been expended to first deny the existence of Gulf War Illness, and then when this proved difficult, to deny that it could be caused by an infectious agent.[3] Most military physicians were content to believe that Gulf War Illness was a psychological problem, not one caused by exposure to biological, radiological or chemical events.[2,3]

One of the interesting stories that occurred during the discovery of an unusual pathogen in approximately 40% of Gulf War Illness patients was that this same agent was present in the Texas prison system in the mid-1970s to late-1980s, well before the Gulf War in 1991.[4] This pathogen, *Mycoplasma fermentans*, is a primitive cell wall-deficient bacteria that was first

patented by a U.S. Army pathologist and was thought to be involved in a number of chronic diseases.[5] In fact, the discovery and most of the research on *M. fermentans* was conducted by U.S. Army laboratories. For example, *M. fermentans* was found by Lo and his collaborators to cause a lethal infection in non-AIDS-infected military personnel.[6]

Until after the first Gulf War the U.S. Department of Defense's own medical school (Uniformed Services University of Health Sciences at the Bethesda National Naval Medical Center) taught the following about *M. fermentans* infections.[7]

> ***Mycoplasma fermentans***[7]
>
> The most serious presentation of *M. fermentans* infection is that of a fulminating systemic disease that begins as a flu-like illness. Patients rapidly deteriorate developing severe complications, including adult respiratory distress syndrome, disseminated intravascular coagulation, and/or multiple organ failure.
>
> The organs of patients with fulminant *M. fermentans* infections exhibit extensive necrosis. Necrosis is most pronounced in lung, liver, spleen, lymph nodes, adrenal glands, heart and brain. M. fermentans is identified in areas of necrosis, particularly in the advancing margin of necrosis, by the immunohisto-chemistry using specific anti-*M. fermentans* and *M. fermentans* incognitus antibiody and/or by in situ hybridization assays using cloned incognitus strain DNA. Mycoplasma-like particles are found intracellularly and extracellularly by electron microscopy.

Microorganisms such as *M. fermentans* have also produced progressive chronic illnesses in a variety of animal species. For example, Lo *et al.*[8] found that injection of *M. fermentans* into nonhuman primates resulted in development of a fulminate, fatal illness. These animals displayed many chronic signs and symptoms but did not mount an antibody response until they were near death.[8] In humans, infections like *M. fermentans* have been associated with increased severity of signs and symptoms in fatiguing illnesses,[9] neurodegenerative diseases,[10] neurobehavioral diseases,[11] autoimmune diseases,[12] complex infections like Lyme Disease[13] and other diseases.[10,14]

Our hypothesis is that this process did not occur randomly. Infections like *M. fermentans* were not acknowledged in the medical literature before the late 1980s. In addition, new emerging illnesses like the fatiguing illnesses (Chronic Fatigue Syndrome, Fibromyalgia Syndrome, Gulf War Illnesses, etc.) were relatively unknown before the 1980s, or were found in only a few cases reported by the 1990s. Some diseases (for example, neurodegenerative, neurobehavioral and autoimmune diseases) showed striking increases in incidence during this same 1980s period.[10] All of these diseases have one thing in common—the high incidence of chronic infections that were rarely found previously.[10] Prominent among these chronic infections were infections caused by *Mycoplasma* species and similar bacteria.[10]

'Weaponization' of New BWMD Agents

Once new biological agents have been isolated and tested in animals and humans, they have to be 'weaponized' before they are considered useful as BWMD. This process must take into account various properties that are necessary for enhanced survival, dissemination and pathogenic properties of the microorganism.

Before the advent of genetic engineering, microorganisms were 'weaponized' mainly by a slow process of selection to obtain variants that possessed the properties necessary for a BWMD agent. For example, the candidate microorganism must be resistant to heat, sunlight and dryness; thus variants were selected for enhanced resistance to these environmental conditions. They also must have strong pathogenic properties and cause sickness by the entry of small numbers of microbes into the body. This could be accomplished by passing the candidate microorganism through multiple animals or even humans to select variants with increased pathogenic properties.

By the 1980s the use of genetic engineering allowed researchers to bypass lengthy selection regimens by inserting particular genes into candidate microorganisms to 'improve' their BWMD properties (Table 2).

Table 2. Some Genes Useful for 'Weaponizing' Candidate BWMD Agents

'Weaponsization' Property	*Gene to be Inserted*	*Resulting Characteristic*
Heat resistance	Thermal resistance genes	Survives explosions or fires
Dry resistance	Spore-forming genes	Survives desert or dry conditions
Cellular entry	Receptor gene	Increased cell entry
Cellular death	Apoptosis or toxin genes	Increased cell and tissue death
Tissue targeting	Tissue receptor gene	Targets particular tissues/organs
Immune resistance	Immune suppression genes	Immune suppression
Mass production	Growth genes	Grows easily to high density in vitro

For example, we hypothesize that 'weaponization' of *M. fermentans* probably occurred by the insertion of several genes that enabled the micoorganism to better survive and possess increased pathogenic properties. Evidence for this genetic process can be found in the genetic signature of *M. fermentans* seen in military patients and those civilians used for experimental studies on the infectious process.

A contentious finding of ours identified a gene from the HIV-1 virus in some military patients infected with *M. fermentans*. The particular gene was the *env* gene, which encodes gp120, part of the viral spikes that allow the HIV-1 virus to bind to specific receptors on cells and allow the virus to enter cells.[15] This is shown in Figures 1 and 2. We hypothesize that insertion of the *env* gene increased the pathogenic properties of *M. fermentans* by allowing it to more easily enter cells as well as target cells of the immune system, just like the HIV-1 virus.[16]

Interestingly, when we examined some of the guards who worked in Texas prisons implicated in the testing of *M. fermentans* as a new BWMD agent (see next section), we found evidence of various HIV-1 genes in different patients (*env, pol* and *rev*) not just the one gene (*env*) found in military patients. This 'exposure' to various gene-containing *M. fermentans* strains preceded the first Gulf War, so it implicated the Texas prisons in the testing of various candidate gene-modified *M. fermentans* mutants for their properties, including their pathogenecity.

Figure 1. The genomic and physical structures of the HIV-1 virus

Figure 2. A hypothesis on how the HIV-1 env gene increases the entry rate and pathogenic properties of Mycoplasma fermentans.

Testing of 'Weaponized' BWMD Agents

Once new candidate BWMD mutant microorganisms have been developed, there is natural pressure to test the new mutants, along with their engineered genomes, to see if their resulting properties make them more effective as BWMD agents. Such testing can be conducted in animals, but for some purposes only humans are useful as test subjects. Thus there is pressure to conduct clinical trials using human subjects. Two of the most useful groups of possible subjects for such clinical trials are military personnel and prisoners.[18]

In the case of *M. fermentans* we believe that clinical testing was performed on both military and prison personnel (inmates). Evidence for this includes the presence of *M. fermentans* in the blood of Gulf War Illness patients who received multiple military vaccines during deployment and in some cases their immediate family members (but only after the veterans returned to the home)[17] as well as in prison guards who worked in selected prisons in Texas.[4] In the case of veterans of the first Gulf War, there was a strong association of Gulf War Illness symptoms to the military vaccines received during deployment.[19]

In the case of prisoners as experimental subjects, Texas has a long history of using prisoners for clinical trials.[18, 20] According to the minutes of the Texas Department of Corrections Board,

selected prisons in East Texas were used for experiments that were conducted using various *Mycoplasma* species to determine pathogenicity and countermeasures against infection.[21] This likely resulted in the spread of mycoplasmal illnesses from prisoners to guards and other prison employees and then to their immediate family members in the community.

In the 1980s in the city of Huntsville, Texas most of the employment was at the local state prisons. In this small East Texas town there was a dramatic increase in neurodegenerative, autoimmune and fatiguing illnesses that were linked to employment at local prisons. Although unproven, it is likely that the increases in chronic illnesses in towns like Huntsville were indirectly linked to the clinical trials being conducted at local prisons.[22] It is very likely that other states also provided experimental subjects from their prisons and military bases for the testing of new or existing BWMD agents, and this may have contributed to the overall increases in the incidence of chronic illnesses seen in various regions around the nation.

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