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Vulnerability to a Bioterrorism Attack and the Potential of Directed Evolution as a Countermeasure

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Review Article

Abstract

This article has two purposes. First, the consolidation of facts about natural and manmade biological warfare agents as well as the use of technology in the development of biological weapons, including near-term future evolvement in genetic engineering to prevent the potential havoc-wreaking events using biological warfare. Secondly, this article aims to suggest steps to tackle the vulnerability of humans to biological warfare and to provide a potential solution that incorporates Directed Evolution to attack the problem of biological weapon proliferation.

Keywords: Biotechnology; Biological weapons; Poisons.

1. Introduction

Technology, it appears, is a double-edged sword. Express advancements in biotechnology, biomedical sciences and bioinformatics are incontrovertibly solving a multiplicity of human problems, but it seems that they are leading to as many troubles as they are intending to settle. At one extremity is the conception of breakthrough drugs against cancer and HIV, and at the other is the inadvertent or on purpose development of more dangerous biological poisons. While problems like non-uniform access and availability of food is gradually giving way to food security all over the world, nonetheless, the term 'Biological Weapon' creates insecurity in the mind of any well-aware human.

Biological warfare can be seen in very simple terms as the use of bacteria, viruses or other toxins (biological warfare agents or biohazards) to cause destruction of men, or animals and plants that serve as source of food for them, or even to the ecology of a place. If this is done in order to cause a devastating feeling of anxiety or fear among sufficiently large populations, generally occupying contiguous areas, it is called biological terrorism or bioterrorism. The biological warfare agents (biological weapons) fall under the category of weapons of mass destruction (WMD), under which also fall the dreaded chemical weapons and nuclear weapons. However, not all reported events involving biological agents are linked with a WMD that is being intended to target larger areas and intended to cause many casualties [1].

2. A Brief History

The use of biological agents as weapons to create disorder in established organizations and institutions, such as administrations and particularly bigger metropolitan populations, has been prevalent all through the course of history. In the last few decades, a noteworthy rise has been seen in the use of biological agents in order to disseminate such substances to cause detriment to the public [1]. The potential danger of *Bacillus anthracis* as a biological weapon was vividly demonstrated after an unintentional release of its spores in 1979 from a military microbiology facility in Sverdlovsk, Union of Soviet Socialist Republics, that resulted in at least 77 cases of human anthrax and 68 deaths [1,2]. Although commonly termed "bioterrorism", the purposes of such attacks are not necessarily intended to intimidate established government structures, but can also be motivated by religious, political, or ecological ideologies [2].

3. Potential of Naturally Occurring Biological Poisons

Potential natural biological warfare agents are the Ebola, the Venezuelan Equine Encephalitis virus, the Marburg virus and the like [3-5]. In more recent times, the primary biological warfare agents that have been reported are anthrax (*Bacillus anthracis*) and ricin, a non-contagious agent that is an albumin, and which can be obtained from *Ricinus communis* (castor bean plant) [2]. Not all of them cause a fatal infection, and are fought and defeated by the natural immune system of the human body. And many of these that have the potential to cause fatal infections have a fixed cure, though, for biological agents like ricin, there are no available treatment options with the exception of supportive care [1]. Obtaining the rarely occurring ones among the rest is difficult. Access to natural and highly virulent agents and strains like the smallpox virus, has become much regulated

and exceedingly restricted even for laboratories [3]. In spite of all these facts, groundwork for a possible bioterrorism attack is tremendously necessary. In case of Woolsorter's Pneumonia (anthrax), because there is no mechanism to discover a premeditated, secret release of the spores of the causative agent, the only step that appears to be exceedingly effective in combating the disease is increased clinical suspicion and vigilance based on knowledge of and insight into the pathogenesis of *B. anthracis* [6]. Similarly, in case of most of the other potential biological weapons, ways of knowing about a deliberate attack have not been identified. Indeed, the real attribute of a potential biological weapon that is chiefly utilized by those who wish to clandestinely undertake an offensive using such agents is the mortality caused by the agent before it is known that the populace has been infected with it. The fact that almost all of the potential biological weapons have the attribute of quick extensive spread among populations and utter difficulty of diagnosis of the diseases they cause makes them the most deleterious among the Weapons of Mass Destruction.

Ebola, if contracted naturally, can cause death in not a very large number of cases [4]. And those who can afford and access available treatments have a creditable probability of survival, assuming that the aim of terrorists is to create terror through deaths since treatment of the diseases they intend to cause may almost negate their efforts to create affright, even though no specific treatment for Ebola in the form of a vaccine is available, and drugs for the same are in the process of being tested or are being considered for being tested, as per WHO [7]. Similar is the case of H1N1 (swine flu), the difference there being that a potential cure was developed within a short time of the occurrence of the pandemic [8, 9], due to the rapid evolution of medical science, where new discoveries are made in the wink of an eye, and inventions occur in leaps and bounds. But susceptible groups like children, already sick people, old persons, pregnant ladies, etc., have amply heightened risk of mortality in both the cases, and that is sufficient to cause terror.

3.1 Comparison between the homicidal potential of chemical and biological poisons

A biological weapon cannot kill as quickly as a chemical weapon can. A biological warfare agent encounters huge resistance from the human body's immune system before being able to work its way out to cause its impact by means of appearance of symptoms. A chemical agent, developed to destroy life, can cause deaths in seconds by the annihilation of living cells or other subtler means. In contrast, a biological poison like the HIV, a retrovirus, can stay in the body for as long as twenty years before its first symptoms appear. Even after that, a person may live for up to a few decades by the way of using anti-retroviral therapy, even though no specific cure for its infection exists [8-11].

But HIV is not a potential biological weapon. It cannot spread extensively in a short duration. The potential biological warfare agents are chosen such that they act very rapidly. Even though, as per what is known about them their lethality pales before that of chemical weapons and they cannot act as quickly as a chemical warfare agent, difficulties related to identification of the diseases they cause render them much more detrimental than the latter.

3.2 Artificial biological weapons and modified natural agents

The creation of a biological weapon in a lab may turn out to be an absolutely onerous task. Not only that, even the most unknown microorganisms, be them viruses or bacteria, may respond to random anti-viral therapies or anti-bacterial therapies as may be the case, including broad-spectrum antibiotics or a combination of them [12]. It is known how to decipher genomic sequences (owing to the research efforts of double Nobelist Sanger), and we also possess the capability to synthesize these chains (through the studies conducted by Nobelist Hargobind Khorana) [13-15]. Thus, creating or altering the DNA or RNA sequences at will, or to perform site-specific mutagenesis (because of the research carried out Nobel Prize winning scientist Michael Smith in this direction) are tasks that can now be easily realized [13,14]. Beginning with the genome of an existing virus, in order to recreate the genome of a microbe, the sequence needs to be available on the internet or, at least, in the books. If it is available, it needs to be assembled by combining shorter DNA or RNA sequences that are already accessible, like what was done by Cello et al. in 2002 [16]. It then has to be modified to add the required genes in it, so that one can get a virus of the particular characteristics demanded by an efficient biological weapon. The protein coat may also be created in the lab, beginning with a prototype and evolving it bit by bit. These stepwise modifications may be carried out with the assistance of Directed Evolution [17,18]. Creating long genome sequences from scratch is burdensome.

If a protein coat with the required characteristics cannot be fashioned in the laboratory, the genome created can be inserted into the protein coat of a virus with its own genetic material removed. One has to take under consideration that environmental robustness depends on the genes of the pathogen along with the attributes of the protein coat. Procurement of suitable virulent strains of micro-organisms, easy multiplication and mass production without the loss of potency and pathogenicity (which also depends on the genes of the microbe), and the development of an effective medium of delivery are quite complex problems, the last one particularly so, and, on the top of that, only few well-trained and commendably proficient experts can bring about the successful culmination of the process [3,12]. Even then, credible evidence suggests that sufficiently erudite scientists are available in plenty numbers,

and are lending their support, to both the clandestine biological weapons programmes of various nations and to the terrorist organizations of the world who wish to succeed in this arena of mayhem.

The USSR's 'invisible anthrax' can be quoted as a crude example of a genetically modified biological warfare agent. The causative vehicle of the disease, *Bacillus anthracis,* had a foreign gene imbibed into its genome which modified its immunological properties, rendering existing vaccines ineffective against its infection [19]. It fulfilled almost every one of the requisite specifications of a biological weapon, viz., being able to be cultured and thus created in large quantities, being able to act rapidly, being environmentally hardy and being vaccine treatable in such a manner that an affected enemy does not have a vaccine or a drug as a cure, but the allies can be protected from its overwhelming impact [3].

But, since the early years of the current millennium, several ways to combat anthrax have been developed. Therefore, for the present generation of terrorists and other such antihuman agents, deploying anthrax as a bioterrorism instrument is almost infeasible since there are limitations with the anthrax bacteria, if the actual aim is to terrorize through destruction of human lives. Its victims can be treated using antibiotics several days after the infection, and only a small minority of those infected die, and those deaths too, can be prevented if awareness has been generated among people about this [3,6,20]. So, it could be seen as a bioweapon that is bound to fail as a mass killing machine, but might be employed to weaken enemy troops during combat.

Despite that, the development of modified anthrax bacteria can be seen as a breakthrough in the evolution of modern biological weaponry, for its creation has showed that a simple genetic change can produce very deadly results indeed. Since *Bacillus anthracis* has the potential to be employed as an agent of bioterrorism, an agent which can swiftly develop into systemic anthrax with high mortality among the persons who are exposed and go untreated, clinical guidance that can be rapidly disseminated as well as implemented must be prepared to preempt any deliberate release of the bacterium [21]. Further, to combat genetically engineered anthrax bacteria, gene therapy shows immense potential. The Human Genome Project shows great promise to have an intense constructive impact on the way biomedical research is conducted and aid in explaining the most unexplained and intricate life processes [22]. Latest techniques in biotechnology should permit the scrutiny of the events that occur in a human cell following the infection by a pathogen or the ingestion of a toxic molecule, which can help clarify the situations that result in individual vulnerability to infectious diseases [12]. Studies in functional genomics may be able to explicate mysteries that still exist regarding the human genome and facilitate the excogitation of new strategies for prevention and treatment of infectious diseases in the form of vaccines and anti-microbial drugs, respectively [12]. Researchers have developed innovative and exceedingly promising gene-editing tools like CRISPR, which can potentially be used to build up effective techniques to combat this disease by rendering humans immune to it through genetic enhancement or eugenics. Immunotherapy, also, can be possibly utilized for this purpose in combination with gene-editing, or otherwise in a standalone manner.

Molecular biology can enable the scientists and the researchers to develop new weapons, which are much more efficient and useful than the natural ones or previously existing ones [3,12]. One of the techniques under the subject can involve the introduction of antibiotic resistance by altering genomes of microbes, which can render them typically noxious biological warfare agents [3]. The human smallpox virus has the potential of being a model bio-weapon, especially for terrorists, because it is decidedly infectious and has the capability to bring about tremendous fatality rates. Nearly the whole of the latest generation among the existing population of the world has not been vaccinated against that particular virus [3,23]. It is horrible to imagine what will happen to the youth if criminals get hold of the smallpox virus somehow and hand it over to wretchedly desperate non-state actors who are capable of turning it into a catalyst of cataclysm.

Fortunately, nowhere is its genome existent except in two chosen highly secure laboratories around the world. Its genome is very big, having about a hundred and ninety thousand base pairs, and is not easily available in the form of data or diagrams [24]. Then again, the polio virus has a much smaller genome (about seven thousand and five hundred nucleotides) which been whose assembly has already been demonstrated by Cello et al. [16] and Kitamura [25]. All the same, a related virus like cowpox, or even monkey pox, can be utilized so that one can mutate and thereby change certain bases and sequences in their genome in such a way that they resemble the human smallpox [3]. Rosengard et al. [26] in 2002, have shown that the sequence of a gene related to the vaccinia virus's pathogenicity, can be transformed with the help of targeted mutation of a small number of base pairs into the sequence of the corresponding gene in the human smallpox virus [26]. But trying to repeat the process to obtain the whole genome of the smallpox virus can possibly only be fantasized, for it sounds to be an exceptionally complex, lengthy and cumbersome process.

3.3 Tackling the vulnerability through socio-political means

We have divided the focus areas for tackling vulnerability, where we particularly lack in skills at the present moment, and those which we deem to be most important, into two: those which should be undertaken during the preparation phase and those which need to be carried out during emergency response phase.

In order to reduce the vulnerability of the society to the biological agents during the preparation phase, among whatever others have prescribed, the focus must be on developing techniques regarding:

1. Quicker and simpler diagnosis of diseases, particularly the emerging infectious and non-infectious diseases, and
2. Immunization against probable natural as well as, to whatever extent possible, manmade or genetically modified biological warfare agents

During the emergency response phase, among whatever other studies have recommended, the focus must be on developing methods for:

1. discovery of the dissemination of a biological agent, if at all an agent has been disseminated, and the method as well as the agency of its dissemination
2. speedy detection of the attributes of the agent deployed, and, utilizing that information, quickly formulating a treatment protocol, and, if possible, a cure
3. inoculating the maximum possible people in the region affected in the minimum possible time through deployment and redisposition of well-protected response teams

These areas have been listed as per what we find significant to ponder. There may be more as well, that need to be taken care of. The ways of discovering the dissemination of an agent have been given a status of critical importance by Grundmann, who suggests that sentinel air-measurement devices in large urban areas or epidemiological investigations are necessary to kick off the response against a bioterrorism attack [1].

The most likely perpetrators of a possible bioterrorism attack are radicalized groups that aim to utilize biological warfare agents to cause mass casualties. Preventative measures by agencies involved against them concentrate on hunting down and disrupting the activities of such groups from obtaining and using biological agents. Most international groups are likely to collaborate with insiders who may at as their proxies, though, who are located in the country where the attack is aimed to take place [1,2]. With the knowledge that the powerful militant groups like the Daesh (ISIS), some of whose intentions include destruction of those who do not conform to their professed ideologies, have transnational targets and multinational presence, and also the existence of the rumors as well as reliable intelligence reports suggesting their setting up of new, and gaining control of old, laboratories– the armories of death – that are suitably equipped to create artificial biological weapons – the ammunition of upheaval, it is high time that the government takes a proactive stance against the possible deployment of these weapons against civilians and food supplies, along with environmental concerns that arise from the use of such weapons.

Funding towards the development of vaccines and treatment options to tackle the dissemination of a biological agent has till now chiefly been concentrated on anthrax, tularemia, smallpox, and ricin, while research projects that aim to create a remedy against a possible bioterrorism attack by other biowarfare agents have been relatively underfunded [1]. The next section shows how a new scientific technique can potentially be employed to counter bioterrorism using vaccines and drugs.

In guise of preparing anti-weed and anti-pest biological agents that might help in agriculture, or developing drugs for the benefit of humans, or under other aliases, various labs in different countries are reportedly developing biological weapons of varying modes of operation and dissemination, and deadliness. Strengthening the various safeguards like those provided under the Biological and Toxin Weapons Convention can go a long way in tackling dangers posed by these weapons.

4. Suggestions

Advances in creation of new vaccines against a number of meningitis and pneumonia bacteria have been made possible as a result of the availability of genome sequences of numerous pathogens [27]. Attempting to develop new vaccines that would stimulate immunity against a multiplicity of diseases with a single treatment, scientists have genetically engineered viruses [12,27].

There are possibilities of developing completely novel weapons on the basis of knowledge provided by biomedical research [3]. But, at the same time, Directed Evolution, in a manner similar to the way it can be involved in the development of bioterrorism agents, can be used to efficaciously counter their impact. For example, in case of genetically modified *Bacillus anthracis,* the analysis of the DNA sequences of all available variants of the anthrax pathogen may be carried out with the help of a database library, and particular regions in the sequence that are preserved in most of the variants may be found. (The sequences in the DNA that are common among all individuals of the species of the bacterium, that in fact render it a unique species, may be considered here.) Such regions can thus be considered as the chink in the armor of most, even if not all, of the *Bacillus anthracis* subtypes known hitherto. Using Directed Evolution, if, step by step, the scientists are able to develop some biocatalyst that has the capability to sever any of these regions from the genome of the bacterium, or, utilizing one or more of those regions, develop a vaccine in stages, where each iteration results in a betterment over the preceding stage, success might be achieved against possible anthrax outbreaks that may occur in case of an attempted bioterrorism assault [28].

But a problem may occur if very limited number of such regions is discovered and these regions are known by those who are in process of developing

biological weapons using genetically modified *Bacillus anthracis,* for they may modify these regions as well; rendering newly developed and effective drugs or vaccines that target those regions, useless. Further, the mission might encounter a roadblock in another way – if no such region is found out. But there is still hope, since the genome of the bacterium is long, the probability of finding such regions increases, and the chance that all these regions are discovered and modified by those who intend to create biological weapons decreases.

In case of those microorganisms whose genome has suspected to have been completely artificially synthesized in the laboratory, genes – that can possibly be incorporated in its genome to render to it the attributes that make the microbe a potential biological weapon – can be brainstormed by scientists and vaccines can be developed against such genes in the manner already described above. Although this mechanism has its drawbacks, it has the potential to not only help in battling the threat of biological warfare agents to a substantial extent but also increase the security and confidence of the people at large. This technique can be replicated against known and possible causative agents of zoonotic diseases. Such researches must be carried out covertly.

5. Conclusion

Directed Evolution may prove to be a decidedly potent tool to counter biological weapons in the near future. Reemphasizing the axiom that technology has its light and dark faces, any technology that is used for a maleficent purpose, the same technology can also be used to counteract against the ghosts which it created. To prevent the technology from being applied for injurious purposes, ultimately, it is the human outlook that has to change from self-centered to selfless, from artificiality to innateness, and from inhumane to compassionate.

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