Review Article

Bioshock : Biotechnology and Bioterrorism

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Abstract

In the recent past, the threat of a global bioterrorist attack has increased dramatically. In addition to the already existing microorganisms and techniques, the recent explosion in biotechnology has considerably added to the arsenal of the bioterrorist. Molecular technologies are now available which can be used by committed bioterrorist groups to manipulate and modify microorganisms so as to make them increasingly infectious, virulent or treatment resistant for causing maximum casualties. Infectious diseases which are likely to be used as bioweapons are Anthrax, Botulism, Plague, Smallpox and Brucella. Molecular techniques like immunoassays and nucleic acid amplification are now available to detect bioattacks. This article discusses the threat of bioterrorism. It also evaluates the molecular diagnostic methods and the future of early containment of a bioterrorist attack using molecular techniques.

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Introduction

The earlier belief that bioterrorism is not a serious threat has been proved wrong [1]. It is evident from the recent attacks that bioterrorism is not a myth but a reality [2, 3]. Biotechnology can be used by committed terrorist groups to produce microorganisms that are capable of large scale morbidity and mortality.

This article briefly outlines the organism attributes and the role of biotechnology in assisting the bioterrorist to produce lethal microorganisms. The converse side of the picture is also discussed i.e the role of the laboratory in detecting, isolating and containing the microorganisms.

Organism Attributes

The five basic attributes that characterize a perfect military biological warfare (BW) agent have already been identified [4]. They are as follows:

a) High virulence coupled with high host specificity

b) High degree of controllability; the organism should attack only specific groups or populations of people and should not attack the people initiating the bioterrorist attack

c) High degree of resistance to adverse environmental forces

d) Lack of timely counter measures to the attacked population

e) Ability to easily camouflage the BW agent.

Some of these attributes might not be so important for BW agents that will be applied for terrorist purposes. For example, a terrorist group might be unconcerned whether or not the agents it uses can be controlled after release. Nevertheless, these criteria serve as useful considerations regarding the type of microorganisms which can possibly be used by bioterrorists. In addition, to develop perfect bioterrorist agents, modern biotechnology techniques may be applied to enhance any or all of eight characteristics or traits of microorganisms i.e. hardiness, resistance, infectiousness, pathogenicity, specificity, detection avoidance, senescence and the viable but non-culturable state [5].

Use of Biotechnology in Enhancing Bioterrorist Weapons

The explosion of knowledge in molecular biology stems from three main discoveries. These discoveries were the discovery of DNA structure, the polymerase chain reaction and the human genome project. The initial discovery of the structure of DNA by Watson et al [6] paved the way for the discovery of the polymerase chain reaction [7]. This in combination with the human genome project allowed scientists to copy, mutate, sequence and manipulate DNA. In addition to the human genome, the sequences of several other microorganisms are freely available on the Internet. This knowledge of molecular biology and genomic structure has helped greatly in the construction of dangerous pathogens.

In 2001, Australian scientists manipulated the

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mousepox virus to suppress the wild mouse population [8]. The outcome was a modified virus that was far deadlier than the original one. This modified strain was also capable of killing mice naturally immune to mousepox or those immunized against the mousepox virus. Since the smallpox and the mousepox viruses are analogous to each other, it is entirely possible that the same experiment can be carried out in the smallpox virus. The smallpox virus is not readily available to terrorist organisations, however it is possible for them to modify other viruses to subvert the human immune system. Again, it is not impossible to synthesize a new organism. In 2002, scientists in USA were successful in synthesizing polio virus from scratch using chemicals available in the open market [9].

Bacteria, mycobacteria and viruses are prone to genetic manipulation. In an attempt to understand why tuberculosis remains latent in some infected individuals, a group of researchers described the creation of a hypervirulent mutant strain of tuberculosis [10]. Genetic manipulation brought out a strain that side stepped the mouse immune system. Similar experiments have been carried out with protozoa like *Leishmania major* [11]. This only goes on to prove that manipulation of known microbiological agents is not in the realm of science fiction any more. Microorganisms can be modified to be more pathogenic or to weaken the host immune system so that they can proliferate and create an uncontrolled infection.

The above paragraphs briefly outline how it is possible to create lethal microorganisms using easily available methods. It would be wrong to assume that the methods would be limited to research laboratories. Most of the techniques used are easily available and can be reproduced in the average laboratory.

The Molecular Basis of Detection

It is easy for the bioterrorist to manipulate the microscopic world for his benefits. However, it is equally easy for the biotechnologist to detect the organism and institute appropriate actions. There are still some challenges which are unique to bioterrorism and others are common for all testing situations. Ideally, detection platforms should be capable of rapidly detecting and confirming biothreat agents, including modified or previously uncharacterized agents, directly from complex matrix samples, with no false results. Furthermore, the instrument should be portable, user-friendly and capable of testing for multiple agents simultaneously. Such an instrument is yet unavailable.

Detection assays must be sensitive and specific, capable of detecting low concentrations of target agents without interference from background materials. In general, nucleic acid-based detection systems are more sensitive than antibody-based detection systems. The polymerase chain reaction (PCR) assay can detect 10 or fewer microorganisms in a short period of time [12, 13]. However, PCR requires a clean sample and is unable to detect protein toxins. Anticoagulants, leukocyte DNA and heme compounds in blood inhibit PCRs [14]. Furthermore, cultures of the target organism are not available for archiving and additional tests after PCR analysis. The high sensitivity of the test can also be a major weakness because contaminating or carryover DNA can be amplified, resulting in false-positive results. This occurs because of operator error, contamination by environmental pathogens and carryover of DNA from previous reactions because of inadequately cleaned instruments.

Quantitative real-time PCR (Q-PCR) combines PCR amplification with simultaneous detection of amplified products based on changes in reporter fluorescence proportional to the increase in product [15, 16]. The main Q-PCR format used for bioterrorist agents is specific target detection and a wide variety of primer and probe combinations are available from many companies in a multitude of configurations. Q-PCR can be utilized to detect several targets simultaneously using different reporter dyes for different targets.

However, accurate characterization or identification of bacteria by Q-PCR is limited by the same bias and variations that are inherent in many nucleic acid techniques. The main concerns are biased nucleic acid extraction (e.g, efficiency of extraction or cell lysis if using whole-cell methods), degradation of nucleic acids by nucleases, probe and primer reactivity (i.e., sensitivity, specificity, accessibility and quantitation), and inherent PCR bias (e.g, variances in polymerase, buffer and thermocycler performances). The ability to either extract the DNA or rupture the cells or spores for accessibility significantly influences the sensitivity, reproducibility and accuracy of any PCR based biothreat agent detection method. Additionally, the presence of inhibitors can interfere with target sites of the probes and primers, thereby resulting in false negatives.

In spite of the limitations, PCR-based analysis can be highly specific and sensitive for the target of interest if the number of infected cells present are at or above the detection limits of the particular assay (typically 10 to 100 cells). Use of Q-PCR to obtain rapid quantitative estimates for biothreat agent presence is an invaluable asset. The new advances in size reduction and speed of thermocycling enable these units to be used both as portable and as laboratory-based platforms.

Immunoassays have increasingly been used and developed for detection of infectious diseases [17].