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## **Biological Weapons: New Threats**

**(Biological warfare and rDNA technology)**

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### **Abstract:**

*The deliberate use of biological agents as weapons has been attempted throughout history. Biological warfare has evolved from the crude use of cadavers to contaminate water supplies to the development of specially designed microorganisms or toxins for future battlefield or covert use. The development of biological agents as potential weapons is accompanied by the advances in basic and applied micro- and molecularbiology. These include first of all the methods of the recombinant DNA technology (genetic engineering) and improved biotechnological processes for the large-scale production of microorganisms. The scientific and technological progress is one of the main reasons for the increased threat from biological agents. Although the idea of making agents with the horrifying feature to attack only specific ethnic groups or distinct human races is rather unrealistic, there are quite a lot of bio-defence research projects dealing with genetically modified pathogens which obviously reveal the potential and capability for the putative development of new biological weapons. However, the recombinant DNA technology is not only the causing of new threats but also the most helpful tool for the establishment of an effective biological detection and early warning system as the central element of future protection against the use of biological agents.*

### **Biological weapons: A historical overview**

Humans have used available technologies for beneficial as well as for destructive purposes throughout history. Attempts to weaponize biological agents such as pathogenic microorganisms and toxins were anticipated by the use of amphibian-derived toxins as arrow poisons.

The first recorded use of biological weapons is illustrated by the outbreak of the plague pandemic in the 14th century due to the siege of Kaffa (now Feodosia, Ukraine). The attacking Tatars catapulted the cadavers of their deceased into the city to initiate plague. An outbreak of plague was followed by the retreat of defending forces and the conquest of Kaffa. Ships carrying plague-infected refugees (and possibly rats) sailed to different Mediterranean ports from where the plague spread out over whole Europe and killed about 25 million people, one third of the then European population. Another documentation of the use of biological weapons was the plan of Amherst, commander of British forces in North America during the French and Indian War in the 18th century, to exterminate the Native

Americans with smallpox. The British forces distributed contaminated blankets and handkerchiefs from the smallpox hospital at Fort Pitt. One of Amherst's subordinates recorded in his journal, „I hope it will have the desired effect“. And it really did. A smallpox epidemic among immunologically naive tribes of Native Americans was the result.

A step towards limiting biological warfare was the 1925 Geneva Protocol. This treaty prohibited the use of biological weapons. However, the treaty did not proscribe research, development, production or possession of biological weapons. Many countries ratified the protocol while stipulating a right of retaliation. Several Parties to the Geneva Protocol started biological warfare programmes after World War I, including Canada, France, UK, and the Soviet Union. The United States ratified the Geneva Protocol in 1975.

From 1932 until the end of World War II, Japan conducted biological weapons research in occupied Manchuria under the direction of Shiro Ishii. Unit 731, a biological warfare research facility, was the center of the Japanese biological weapons development program. Prisoners were infected with pathogens including *Bacillus anthracis*, *Neisseria meningitidis*, *Shigella* spp, *Vibrio cholerae* and *Yersinia pestis*. More than 10'000 prisoners died as a result of experimental infection or execution following experimentation during the Japanese program between 1932 and 1945. In addition, Japan attacked at least 11 Chinese cities with biological agents. Attacks featured contaminating water supplies and food items with pure cultures of *B. anthracis*, *V. cholerae*, *Shigella* spp, *Salmonella* spp and *Y. pestis*. Cultures were also sprayed from aircraft.

During World War II, prisoners in Nazi concentration camps were forcibly infected with *Rickettsia* spp, hepatitis A virus and *Plasmodia* spp and treated with investigational vaccines and drugs. However, these inhuman experiments were done to study pathogenesis and to develop vaccines rather than to develop biological weapons. The Allies, on the other hand, developed biological weapons for potential retaliatory use in response to German biological attacks. Bomb experiments of weaponized spores of *B. anthracis* were conducted on Gruinard Island near the coast of Scotland and resulted in heavy contamination. Viable anthrax spores persisted until the island was decontaminated with a solution of formaldehyde and seawater during 1986. The US program was expanded during the Korean War in the early 1950s. A new production facility incorporating adequate biosafety measures was constructed. Technical advances allowed large-scale fermentation, concentration, storage and weaponization of microorganisms. Production was begun in 1954, whereas a program to develop countermeasures, including vaccines, antisera and therapeutic agents to protect troops from a possible biological attack, started earlier in 1953. By the late 1960s, the US military had developed a biological arsenal that included numerous bacterial pathogens, toxins and fungal plant pathogens. In addition, weapons for covert use using cobra venom, saxitoxin and other toxins were developed for use by the Central Intelligence Agency. All records regarding their development and use were destroyed during 1972.

In 1969/70 President Nixon terminated the US offensive biological weapons program by executive order. The United States adopted a policy never to use biological weapons, including toxins, under any circumstances. It was an unilateral disarmament effort obviously influenced by the fact that biological weapons were then considered untried, unpredictable, potentially hazardous also for the own troops and therefore not suitable for military use. At the same time, there was increasing international concern regarding the indiscriminate nature, the epidemiologic risks and lack of epidemiological control measures for biological weapons, as well as the ineffectiveness of the 1925 Geneva Protocol for preventing biological weapons proliferation. Mindful of the dangers of biological warfare, many scientists urged an international treaty to ban such weapons. The WHO issued a report regarding the potential consequences of biological warfare in which the estimates of the casualty figures that could result from biological attacks were staggering. After disarmament proposals from Great Britain and the Warsaw Pact nations, the 1972 Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction (BWC) was developed. The treaty prohibits to develop, produce, stockpile or otherwise acquire or retain biological agents and toxins in quantities that have no justification for prophylactic, protective or other peaceful purposes.

Despite the implementation of the Biological Weapons Convention, some State Parties to the Convention continued an offensive biological warfare program. The most obvious perpetrator in that context was the former Soviet Union. The Soviets were accused of still developing biological warfare agents after the most worrisome accident in Sverdlovsk in 1979 with a subsequent anthrax outbreak. The US government maintains that the epidemic was caused by an explosion at a biological warfare facility. The Soviets deny the charge, saying that there was an occurrence of anthrax, but that it was due to the sale of tainted meat on the black market. Some Western scientists maintain that the US hasn't proved its allegations. However, it was in 1992 when Russian President Yeltsin himself admitted that the facility where the accident occurred had been part of an offensive biological weapons program and that the epidemic had been caused by a non-intentional release of anthrax spores.

Whereas in 1969 above all the US officials could see no practical use of biological warfare in conflicts, the situation has now changed drastically. New threats from biological weapons arose with the advent of new biotechnology and the recombinant DNA technology and quite recently with the increasing interest in sophisticated, unconventional means and weapons by terrorists. The biological threat posed by terrorists was for example demonstrated by the intentional contamination of salad bars in different restaurants in Oregon with *Salmonella typhimurium* by the Rajneeshee cult during September 1984. This incidence resulted in 751 disease cases and 45 hospitalizations. The threat of biological terrorism resurfaced after the Aum sarin attack on the Tokyo subway system in 1995. Police raids and investigations of the cult's facilities disclosed also evidence of a biological weapons program. The cult was allegedly conducting research with *Bacillus anthracis* (Anthrax), *Clostridium botulinum* and other pathogens. The Aum cult had allegedly launched three unsuccessful biological attacks in Japan using Anthrax and botulinum toxin and had sent members to the former Zaire during 1992 to obtain Ebola virus for weapons development.

The „gene age“ already began in the early 1970s when scientists at Stanford University discovered how to separate DNA molecules by restriction enzymes and then recombine them into a different DNA. From the earliest development of the recombinant DNA (rDNA) techniques, their use for the design and manufacture of agents of biological warfare has been considered. Molecular biologists have long been haunted by worries about creating some new disease and releasing it by accident. The worst visions are those of a disease, against which mankind has no natural immunity, sweeping the world like smallpox or plague. These fears drove US scientists to propose a moratorium on certain seemingly high-risk experiments in 1974, shortly after the first successful recombinant DNA experiments were performed at Stanford University. Public fears were aroused around several centers of rDNA research most notably in the United States, where city officials hotly debated whether to ban all such experiments at local institutions. However, the moratorium was lifted in 1975 at the Asilomar conference in California, when scientists adopted strict laboratory research guidelines which became international standard.

Although, today, there exists a close-meshed net of safety precautions and international biosafety guidelines for the civil application of biotechnology and rDNA techniques, a putative misuse for military and terrorist purposes cannot be excluded at all. On the contrary, the new technologies, including first of all rDNA technology, are certainly the main reason for the believe of an increasing threat from biological weapons today.

### **Threat from bio- and rDNA technology: risk potential**

The following official statements give an idea of the Russian and US assessments of the risk of new technologies with regard to biological weapons:

*„Achievements in biology and related sciences have led to an increase in the effectiveness of biological agents as a means of conducting warfare. Improved methods of obtaining and using them*

*have resulted in a qualitative reexamination of the very concept of biological weapons“ (Soviet Union’s Military Encyclopedia ‘83).*

*„Perhaps the most significant event in the history of biological weapons development has been the advent of biotechnology (including rDNA technology)“ (Statement of the US Department of Defense ‘86).*

With the rDNA technology, it is possible to construct new, genetically engineered biological warfare agents with special properties that make their military use more convenient and efficient. It has been suggested that rDNA technology might facilitate weaponization by rendering microorganisms more stable during dissemination, for example by increasing their resistance to high temperatures and ultraviolet radiation. Biological agents might also be genetically modified to make them more difficult to detect by immunological means and insusceptible to standard vaccines or antibiotics. There is a great fear that, on the one hand, such new biological weapons could be used in future conflicts or, on the other hand, that a genetically modified microorganism for warfare might leap out of control of its inventors, leading to world-wide catastrophe. But even its peaceful applications have engendered fear of genetic accidents unleashing an epidemic of some hitherto unknown disease against which mankind would have no immunity. An uncontrollable pathogenic agent would certainly lead to a horrifying scenario. But there are also other factors that turn biotechnology, and specially the rDNA techniques, into a dangerous tool for the development and production of new potential biological warfare agents.

One of these factors that is less obviously recognized as a risk with regard to biological warfare agents is the increasing knowledge coming from the basic molecular research. There is an increasing number of laboratory groups dealing with the rDNA technology and genetic engineering. In Switzerland, in the past ten years this number rose from 50 to nearly 800 in 1996. A lot of research projects are underway to analyse the mechanisms of virulence, resistance, pathogenicity or other special properties of microorganisms that are of concern for human health. One of the most ambitious projects is the sequencing of the entire genom of the human being. This huge international project is called HUGO where HUGO in fact stands for the organization behind it, namely the Human Genome Organization. The aim of this project is to provide insight into the organization and function of the genetic make-up, and in the course of this work to base physiology and medicine on solid molecular foundations, in order to provide the biochemical basis for understanding hereditary diseases, the mechanisms of immune response and of carcinogenesis such as the appearance of cancer tumours and the like. The genome project has required great effort to build up compatible and accessible data bases to store the information which has been gathered from many different sources and aquired in various contexts by a variety of methods. These data bases will be available to all scientists and may thus constitute a valuable basis for openness about the work done in the whole project but at the same time, this open availability to the information presents also considerable risk of misuse. There are other genome projects that generate a lot of sensitive information, for example the sequencing of the genome of *Francisella tularensis*, a classical biological warfare agent.

Another risk with regard to the potential of biotechnology arises from the growing biotechnology industry and its production capabilities, such as know-how, technology and equipment, most of which are dual-use. The civil microbiological production capabilities can be switched over from the peacetime mission to the production of pathogenic microorganisms. The dual-use character with legitimate applications in commercial fermentation and biotechnology industries is the fundamental problem in countering the proliferation of biological and toxin weapons. Many developing countries have aquired industrial microbiology plants for the production of fermented beverages, vaccines, antibiotics, ethanol (from corn or sugar cane), enzymes, yeasts, vitamins, amino acids and single cell protein as a supplement for animal feed. This global expansion of the civilian biotechnology industry, combined with the growing number of molecular biotechnologists trained in the West, has created much broader access to the expertise and equipment needed for the development of biological warfare agents. Sophisticated laboratories that might be used for the design of novel biological weapons are relatively inexpensive compared for instance with nuclear weapon plants. Moreover, biotechnology and above all the rDNA technique are information-intensive rather than capital-intensive, and much of the relevant data are available in the published scientific literature. For these reasons it is virtually

impossible for industrialized states to prevent the diffusion of weapon-relevant information to states of proliferation concern. It has been estimated that more than 100 countries have now the capability - if not necessarily the intent - to develop at least crude biological weapons based on the available new technology.

### **General principles and possibilities of rDNA technology**

For biological warfare purposes, genetic engineering could open a large number of possibilities. Normally harmless, non-disease-producing organisms could be modified to become highly toxic or produce diseases for which an opponent has no treatment or cure. Other agents, now considered too unstable for storage or biological warfare applications, could be changed sufficiently to be an effective agent. At present, rDNA techniques allow scientists to effect changes in only a small number of genes. In spite of these limitations, it is possible to accomplish three types of modifications or applications:

First, it is possible to increase the resistance of bacteria to antibiotics. Resistance factors can be transferred with new molecular (rDNA) methods relatively easily between unrelated bacterial species. This is a unique achievement that multiplies the possibilities of creating antibiotic-resistant organisms. Second, it is possible to increase or enhance various virulence factors, including the ability of bacteria to produce toxins. Most of the virulence factors are encoded on extrachromosomal DNA, the so-called plasmids which can be isolated and again transferred via rDNA methods to other not necessarily related species. For example, it is theoretically possible to transfer the genes coding for botulinum toxin production from *Clostridium botulinum* to the non-toxic *E. coli*. And third, it is possible to rearrange genes in order to create new microorganisms or at least microorganisms with totally new properties. As an example, the antigenic makeup of an influenza virus could conceivably be altered to the point that it resembles the deadly form that killed millions of people throughout the world in 1917. The influenza virus for instance has sufficient volume to allow scientists to insert for instance the genes that produce cobra venom. And because influenza spreads so easily it may be the best carrier for biological warfare purposes.

The following citation of publications from different research groups in China, Russia and the United States illustrates the possibilities of the genetic engineering and the potential threat from the ongoing rDNA research work with pathogenic agents or parts of them: In 1988 Chinese scientists have isolated the cholera toxin and transferred it into the non-toxic and harmless *E. coli* to study the mechanisms of its activity. They have found that in *E. coli* the toxin has the same bioactivity, antigenicity and immunogenicity as in the original organism *Vibrio cholerae*. However, 90% of the toxin produced is retained within the cells. But then the scientists have found that the toxin can be activated in this situation by the protein degrading enzyme trypsin. In 1992 Russian scientists analysed the smallpox virus with special interest for the proteins that were found to allow the virus to overcome the barriers of host defence against viral infection. Their work was aimed at a better understanding of the host cell defence and protective mechanisms which could be very important for the construction of possible new pathogenic agents. Another Russian research group has used rDNA techniques in 1993 for immunogenicity and virulence studies of *Bacillus anthracis*, the classical biological warfare agent, to be able to produce better vaccines for prophylaxis. They cloned different factors that are important for the toxicoinfection process inter alia into *E. coli*. In the same year US scientists investigated in various experiments the expression of the *B. anthracis* toxin genes. One experiment included the design of a special plasmid with different gene functions that allow to regulate the anthrax toxin production in *B. anthracis*. With the appropriate induction of these functions it is possible to produce unusually large quantities of the toxin. Of course this is a necessary step to study the toxin function and possible protective measures against it, but it could as well be a step in the production of a more dangerous biological warfare agent. Furthermore, Soviet scientists were attempting to recombine the venom-producing genes from cobra snakes with ordinary viruses and bacteria. Such organisms would infect the body and produce paralytic cobra neurotoxin. Although this is quite a delicate study with regard to the development of biological weapons, again, there are good scientific reasons to perform research

work like this. It is supposed that genetically engineered drugs could perhaps kill cancer cells while not affecting healthy cells.

According to these remarks, it's quite obvious that the rDNA technology implies the danger of misuse for the development of new biological warfare agents. There are indeed a lot of possibilities with the new techniques in order to make biological agents more suitable as weapons. There have been suggestions that genetic differences between ethnic groups might be the basis for a new selective biological weapon. Some ethnic groups contain a defective gene or enzyme deficiency in larger numbers of people than other groups do. For example there are some human races that are more likely to have the gene that causes sickle cell anaemia. Other populations have enzyme deficiencies which may or may not inconvenience them. Now it has been suggested that these populations could be targeted in some way with a biological agent which would not affect people lacking the marker gene. However, this proposition remains in the realm of science fiction. Even if it were possible to attack selectively the carriers of sickle cell anaemia genes, it is hard to see the military utility in killing 20% of the appropriate population, which is about the rate at which the gene occurs in the population. In addition, it must be remembered that no race is pure. Casualties might therefore occur in unexpected places.

### **Protection: biological detection systems and new vaccines**

Although the development in basic and applied micro- and molecularbiology can favour the development and production of dangerous biological warfare agents, the set-up of new technologies including rDNA techniques become more and more important for the detection of biological weapons. In the recent past, methods of detection have improved. Nowadays, the detection and analysis of aerosol clouds, biological and non-biological materials or particular agents, even those which have been genetically manipulated, is feasible not least because of bio- and rDNA technology.

Besides the rather classical and already well-known detection systems for military use, such as the Biological Integrated Detection System (BIDS) from the US or the Canadian Integrated Biochemical Agent Detection System (CIBADS), and the new approaches with ion-trap spectrometry and laser-induced breakdown spectroscopy both of which are not directly linked to the bio- and rDNA technology, there are now very suitable molecular methods available for an efficient and very sensitive detection of biological agents. One of these methods is the application of the Polymerase Chain Reaction called PCR which derived from rDNA technology and which is certainly the most valuable tool for a rapid identification and characterisation of pathogenic microorganisms. The PCR is very convenient for the detection of potential biological warfare agents because only smallest amounts are necessary for the analysis. To perform the reaction no precultivation and isolation of the agents are needed. Therefore, PCR allows the direct investigation of crude samples from various origins. This is an important advantage above all in situations where the pathogenic agents are very difficult to cultivate. The field covered by diagnostic PCR is wide, it goes from fungi, bacteria and viruses in environmental or clinical samples to food born diseases where especially genes encoding for enterotoxins can be detected. The importance of PCR as a generally used detection and early warning system for an appropriate protection from biological warfare agents is also shown by the fact that it is discussed within NATO as a future standard method.

Another example from a different branch of rDNA techniques is used for the immuno-prophylaxis against biological weapons. Since 1990 a new strategy for active immunization, the genetic immunization with "naked" DNA, has been established. Accidentally, it was detected that muscles of living mice were able to produce the encoded foreign proteins after direct injection of "naked" DNA. This observation led to the experiments with influenza virus including genes that are able to induce an appropriate immune response in the host. The possibilities of this new method of vaccination are manifold. For instance the DNA vaccination may complement or even replace in the near future the use of some antibiotics getting ineffective due to the increasing spread of antibiotic resistant bacteria. The procedure of DNA vaccination also influences gene therapy, especially cancer therapy. Of course,

also an immunization for the protection against different putative biological warfare agents is under discussion. One of the advantages of that type of vaccine is the relatively cheap production compared to conventional vaccines.

## **Conclusion**

New threats from biological weapons generated by the advances in basic and applied biology are serious and should not be underestimated. It has been suggested that new pathogens created by genetic engineering would be more virulent and harder to protect against than the well-known warfare agents. This is well possible, but it has to be mentioned that the range of pathogens available in nature for use as weapons is already considerable. It would be much easier and, by the way, also much cheaper for a potential proliferator to take some pathogenic agents from endemic places where they occur naturally. The expenses for an rDNA project which would be necessary to develop a new biological warfare agent is estimated at \$50 million over five years, whereas the production of one kilogram of botulinum toxin which can be produced without rDNA technology costs only \$500. One kilogram of this toxin is by far enough to kill the whole population of the world. In that sense the influence from bio- and rDNA technology on the production of biological weapons must see in relative terms. On the other hand, rDNA methods offer very helpful tools for the protection against biological warfare agents. In so far, the applications of bio- and rDNA technology, specially in the military, are not only a potential danger, but also an ability to combat the new threat.