УДК 606:601.4:608.3
https://doi.org/10.35825/2587-5728-2020-4-4-421-430

Today's Biothreats – Where the Past Predictions Meet the Future

Ján Lakota

Centre of Experimental Medicine, SAS, Dubravska cesta 9, 841 04 Bratislava, Slovakia
Faculty of Management Comenius University, Odbojárov 10, 841 04 Bratislava, Slovakia

Received 12.10.2020. Accepted for publication 21.12.2020

The catastrophic spread of COVID-19 pandemic, uncontrolled by modern medical science, regardless of whether it is artificial or not, clearly shows the limits of human mind and knowledge to resist this and other similar challenges. The *purpose of this work* is to show the danger of dual-use biotechnologies in the development of fundamentally new approaches to biological damage to humans. The forecasts of the development of biotechnologies, made by the experts of the American organization JASON and other specialists 23 years ago, are analyzed. It is shown in the article, that in general these forecasts and assessments turned out to be true. The technologies that, most probably, can be used for the development of new means of biological destruction are: binary bioweapons – these are two-component systems that are relatively safe to handle but become deadly when the two components come together on deployment; designer genes – where specific unnatural gene sequences are built into viruses or other life forms to incorporate into the genome of the unsuspecting host, which later becomes the victim; gene therapy – today a medical (partial) reality; the technology that allows medicine to repair or replace defective genes in a diseased individual might be subverted to introduce pathogenic sequences into healthy individuals; stealth viruses – viruses that could be fashioned by a researcher to infect the host but remain silent until activated by some physiological or environmental trigger; host-swapping diseases – new zoonotic agents which might be developed specifically for bioweapon purposes by modifying existing pathogens to seek human hosts; designer diseases – where the detailed knowledge of biochemical signaling pathways could conceivably be used to create designer diseases. In addition to those predicted by JASON, another dual-use technology has emerged recently – synthetic biology. It is a very powerful interdisciplinary branch of biology. Specific examples of the use of these technologies to create new means of biological warfare are given in the article. The author believes that it is necessary not only to track new dual-use biotechnologies, but also to improve conventional and scientific methods of monitoring their use.

Keywords: *binary weapons; biological weapons; biotechnology; CRISPR; disease; DNA; Escherichia coli; gene engineering; gene therapy; pathogens; synthetic biology; terrorism; virus.*

For citation: *Lakota Ján. Today`s Biothreats – Where the Past Predictions Meet the Future // Journal of NBC Protection Corps. 2020. V. 4. № 4. P. 421–440. https://doi.org/10.35825/2587-5728-2020-4-4-421-430*

Biological weapons are referees to those which contain replicating infectious and lethal forms of life including bacteria, viruses, fungi, protozoa, prions, or poisonous chemical toxins produced by living organisms. The use of biological-type weapons is a planned to spread life-threatening diseases on a mass scale in order to devastate the population of an area. In broader sense of the world of biological weapons belong insects which can be used as (biological) weapons against crops, animals and human beings.

In 1960 the US government formed a secret group of academic scientists, the JASON Group mostly having to do with defense. «JASON is an independent group of elite scientists which advises the United States government on matters of science and technology, mostly of a sensitive nature». The JASONs, named after the mythical Jason and the Argonauts – a group of young adventurers who embark on a journey to obtain the Golden Fleece[1]. In 1997 this group addressed the problem of next-generation bioweapons threats.

[1] About JASON Group. URL: https://isgp-studies.com/jason-group-national-security-science; https://web.archive.org/web/20110607181553/http://ist-socrates.berkeley.edu/~schwrtz/SftP/Jason.html; https://fas.org/irp/agency/dod/jason/ (date: 01.10.2020).

In fact, technology over the past 20 years enabled scientists to engineer pathogens to be qualitatively different from conventional bioweapon agents [1]. In terms of bioweaponry, this includes the ability to give these «classic» pathogens attributes that might make them safer to handle, more virulent, more transmissible, harder to detect, and easier to disseminate. In their 1997 report, several broad classes of unconventional pathogens were identified by the Jason Group [2].

These classes include:

i) **Binary bioweapons** (these are two-component systems that are relatively safe to handle but become deadly when the two components come together on deployment).

ii) **Designer genes** (where specific unnatural gene sequences are built into viruses or other life forms to incorporate into the genome of the unsuspecting host, which later becomes the victim).

iii) **Gene therapy** (today a medical (partial) reality; the technology that allows medicine to repair or replace defective genes in a diseased individual might be subverted to introduce pathogenic sequences into healthy individuals).

iv) **Stealth viruses** (viruses that could be fashioned by a researcher to infect the host but remain silent until activated by some physiological or environmental trigger).

v) **Host-swapping** diseases (new zoonotic agents which might be developed specifically for bioweapon purposes by modifying existing pathogens to seek human hosts).

vi) **Designer diseases** (where the detailed knowledge of biochemical signaling pathways could conceivably be used to create designer diseases).

To these classes belong to«modern» or «new» threats which were not mentioned by Jason's – **synthetic biology**. One can define synthetic biology as an interdisciplinary branch of biology combining disciplines such as biotechnology, evolutionary biology, molecular biology, systems biology, biophysics, computer engineering, and genetic engineering [3].

For further discussion we shall take the (well written) parts from the special study on the matter [4]. Although this 16 year old text is little «out of fashion» its originality cannot be overlooked.

Binary bioweapons

«Analogous to a binary chemical weapon, this is a two-component system consisting of innocuous parts that are mixed immediately prior to use to form the pathogen. This process occurs frequently in nature. Many pathogenic bacteria contain multiple plasmids (small circular extrachromosomal DNA fragments) that code for virulence or other special functions. The virulence of anthrax, plague, dysentery, and other diseases could be enhanced by these plasmids. What occurs naturally in nature could be artificially conducted with basic biotechnology techniques in the laboratory. Virulent plasmids can be transferred among different kinds of bacteria and often across species barriers. To produce a binary biological weapon, host bacteria and a virulent plasmid could be independently isolated and produced in the required quantities. Just before the bioweapon was deployed, the two components would be mixed together. The transformation of the host organism back into a pathogen could conceivably take place after a weapon is triggered and during transport/flight» [4].

As an example of (theoretical) binary bioweapon we can use the of so called «EHEC scandal». Novel strain of *Escherichia coli* O104:H4 bacteria caused a serious outbreak of foodborne illness focused in northern Germany in May through June 2011. The illness was characterized by bloody diarrhea, with a high frequency of serious complications, including hemolytic-uremic syndrome (HUS). The outbreak was originally thought to have been caused by an enterohemorrhagic (EHEC) strain of *E. coli*, but it was later shown to have been caused by an enteroaggregative *E. coli* (EAEC) strain that had acquired the genes to produce Shiga toxins, present in organic fenugreek sprouts. In all, 3,950 people were affected and 53 died, 51 of whom were in Germany. 800 people suffered hemolytic uremic syndrome (HUS), which can lead to kidney failure. A handful of cases were reported in several other countries. Essentially all affected people had been in Germany or France shortly before becoming ill. Russia banned the import of all fresh vegetables from the European Union from early June until 22 June 2011. The outbreak was caused by a strain of *E. coli* of the serotype O104:H4, that was unusual for having characteristics of both enteroaggregative *E. coli* and enterohemorrhagic *E. coli*. The strain has a number of virulence genes typical of enteroaggregative *E. coli*, including attA, aggR, aap, aggA, and aggC, and, in addition, to the Shiga toxin variant 2. All bacteria isolated from patients in this outbreak were resistant to beta-lactam antibiotics, third-generation cephalosporins, and partially resistant to nalidixic acid, but susceptible to carbapenems and ciprofloxacin[2]. Paradoxically, a so called «confidential» newsletter on politics and economics («Vertraulicher Schweizer Brief» = «Confidential swiss letter») reported that agents of the German foreign intelligence agency BND (Bundesnachrichtendienst = Federal Intelligence Service) have met agents of other European

[2] 2011 Germany *E. coli* O104:H4 outbreak. See: https://en.wikipedia.org/wiki/2011_Germany_E._coli_O104:H4_outbreak (date: 20.10.2020).

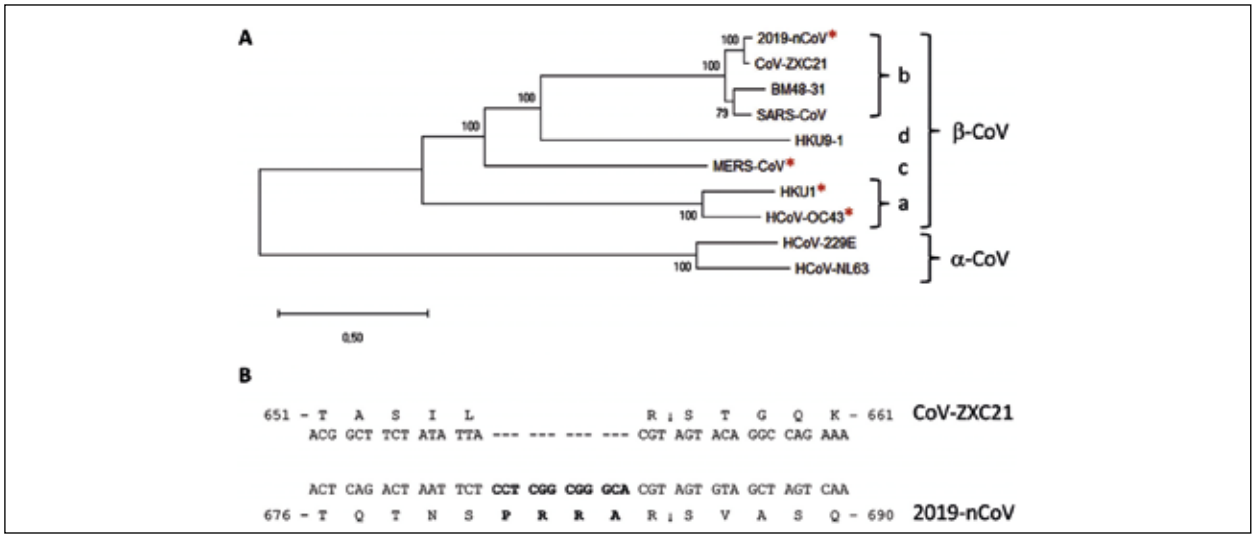

Fig. 1 – *Characterization of an nCoV-peculiar sequence at the S1/S2 cleavage site in the S-protein sequence, compared SARS-like CoV. (A) Phylogenetic tree of selected coronaviruses from genera alphacoronavirus (α-Cov) and betacoronavirus (β-CoV), lineages a, b, c and d: SARS-CoV-2 (2019-nCoV) (NC_045512.2), CoV-ZXC21 (MG772934), SARS-CoV (NC_004718.3), SARS-like BM4821 (MG772934), HCoV-OC43 (AY391777), HKU9-1 (EF065513), HCoV-NL63 (KF530114.1), HCoV229E (KF514433.1), MERS-CoV (NC019843.3), HKU1 (NC_006577.2). The phylogenetic tree was obtained on the Orf1ab amino acid sequence using the Maximum Likelihood method by Mega X software. Red asterisks indicate the presence of a canonical furin-like cleavage motif at site 1; (B) Alignment of the coding and amino acid sequences of the S-protein from CoV-ZXC21 and 2019-nCoV at the S1/S2 site. The 2019-nCoV-specific sequence is in bold. The sequence of CoV-ZXC21 S-protein at this position is representative of the sequence of the other betacoronaviruses belonging to lineage b, except the one of 2019-nCoV [5]*

countries and U.S. agents at an assessment center near Paris to discuss the EHEC outbreak as a potential terror attack of certain North-African Islamic terror groups (especially from Algeria) as some kind of «feces-jihad». EHEC-germs might have been placed intentionally and simultaneously at different market places for foodstuffs, mainly in Germany[3]. However, the validity of this statement remains unproven.

Designer genes

«The Human Genome Project has decoded the alphabet of life and provided a human molecular blueprint. Likewise, the complete genome sequences are now known for 599 viruses, 205 naturally occurring plasmids, 31 bacteria, one fungus, two animals, and one plant. Many of these genomes have been published in unclassified journals and on the internet. To the bioweaponeer these are essentially blueprints that would enable him to make microorganisms more harmful. Now that the codes are known, it seems only a matter of time until microbiologists develop synthetic genes, synthetic viruses, or even complete new organisms. Some of these could be specifically produced for biological warfare or terrorism purposes. Perhaps the most obvious way to increase the effectiveness of any biological warfare pathogen is to render it resistant to antibiotics or antiviral agents. Some bacteria naturally develop resistance to antibiotics fairly quickly. Many antibiotic resistance genes have been identified. The best known of these is the gene that codes for beta-lactamase, the enzyme that defeats the action of penicillin. Such genes could be activated or introduced into other pathogens. Entire viruses may similarly be created, analogous to the natural mutation of the influenza virus. A new strain of influenza could be created by induced hybridization of viral strains, simply swapping out variant or synthetic genes. Slightly altering a common virus like influenza to make it deadlier might be easier than manipulating more rare or biologically complicated pathogens. For a bioweaponeer, the databases of increasing numbers of microbial genomes provide a virtual – parts list of potentially useful genes for a genetic – erector set to design and produce a new organism. It is possible to pick and choose the most lethal characteristics. Some think it may be possible to create an entirely new organism from scratch. Some animal viruses are so small that their entire genome could be stitched together, at least in principle, from machine-synthesized fragments

[3] URL: https://flutrackers.com/forum/forum/the-pandemic-discussion-forum/109441-ehec-outbreak-in-germany-conspiracy-theory-on-potential-terror-attack

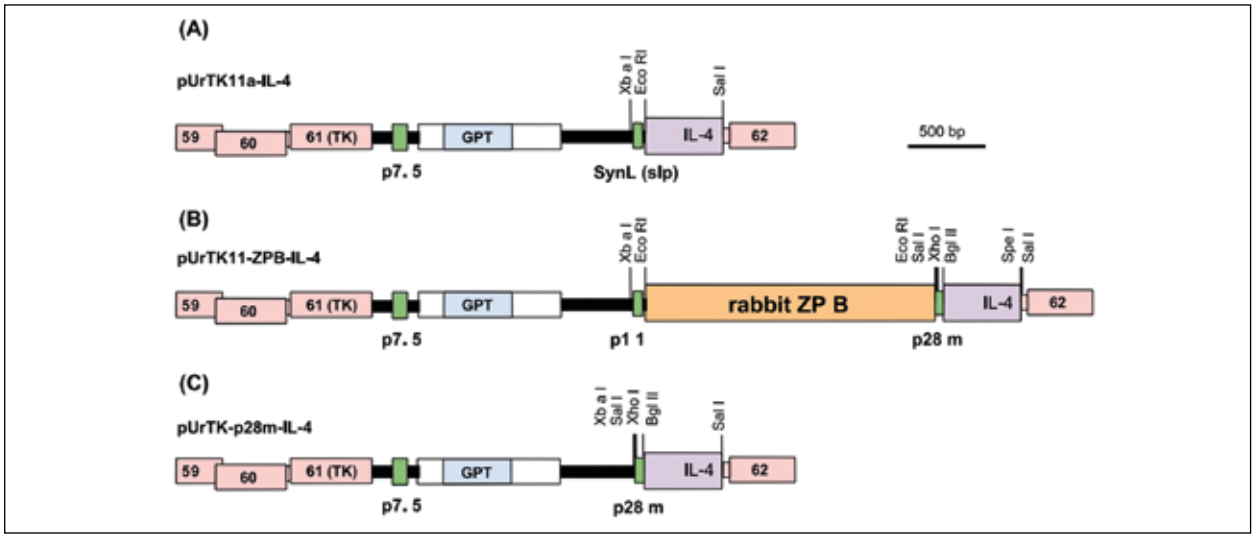

Fig. 2 – *Plasmid constructs used as transformation vectors. The IL-4 cDNA is under control of the strong synthetic late promoter (slp) in pUrTK11a-IL-4 (A), which was used to construct SLS-slp-IL-4 recombinant virus. The IL-4 gene is under control of the modified p28 (p28m) promoter in pUrTK11-ZPB-IL-4 (B), which was used to construct Ur-ZPB-p28m-IL-4 recombinant virus. The IL-4 gene is also under control of the p28m promoter in pUrTK-p28m-IL-4 (C), which was used to construct SLS-p28m-IL-4 recombinant virus. Flanking myxoma virus sequences from the M059R to M062R genes are identified by their abbreviations 59 to 62. TK = thymidine kinase. Unshaded areas in the GPT gene indicate noncoding sequence; black bars around the promoters indicate noncoding sequences from vaccinia virus contained within the background plasmid. In each case, insertion of the foreign DNA will be intergenic between the M061 and M062 genes [7]*

using current technology. Mycoplasma, an organism that causes pneumonia in humans, has the smallest known bacterial genome. Genetic analyses of strains of mycoplasma indicate that only 265 to 350 genes are essential under laboratory growth conditions. Thus, it may be possible to create an entirely synthetic – minimal genome organism in the near future. If a streamlined cell of this type were available, it would be an attractive template to build a bioweapon. As stated previously about viruses, although it may be possible to create life artificially from a set of component parts, this would probably be beyond the sophistication of most bioterrorists. It would be extremely difficult to engineer all of the desired - attributes into a single pathogen and still have an organism that transmitted effectively and predictably. It would be much more likely that an existing pathogen would be subtly genetically modified to be more difficult to detect, more virulent, or more resistant to drugs, all within the capabilities of today's biotechnology» [4].

In the paper: «The spike glycoprotein of the new coronavirus SARS-CoV-2 (2019-nCoV) contains a furinlike cleavage site absent in CoV of the same clade» the authors identified a peculiar furin-like cleavage site in the Spike protein of the 2019-nCoV, lacking in the other SARS-like CoVs [5]. This may have significant functional implications for virus entry. This inserted sequence (CCT CGG CGG GCA) codes for four «new» amino acids (PRRA). Furin-like cleavage site may provide a gain-of-function to the SARS-CoV-2 for efficient spreading in the human population compared to other lineage b betacoronaviruses. The abovementioned paper reached in October 2020, 280 citations (Fig. 1).

Furthermore, the insertion of a multibasic motif RERRRKKR↓GL at the H5N1 hemagglutinin HA cleavage site was likely associated with the hyper-virulence of the virus during the Hong Kong 1997 outbreak [6].

Gene therapy

«Gene therapy will revolutionize the treatment of human genetic diseases. The goal is to effect a permanent change in the genetic composition of a person by repairing or replacing a faulty gene. Genes have already been spliced into bacteria to produce human insulin in large quantities. The eventual goal is to splice a gene that codes for the production of insulin into human pancreatic tissue to cure diabetes. Similar research is progressing on adding in the missing gene to prevent the symptoms of cystic fibrosis. However, the same technology could be subverted to insert pathogenic genes. There are two general classes of gene therapy: germ-cell line (reproductive) and somatic cell line (therapeutic). Changes in DNA in germ cells would be inherited by future generations. Changes in DNA of somatic cells would affect only the individual and could not be passed on to descendants. Manipulation of somatic cells is subject to less ethical scrutiny than manipulation of germ cells.

This concept has already been used to alter the immunity of animals. The vaccinia virus (a poxvirus used to make immunization against smallpox) has been used as a vector to insert genes in mammalian cells. This genetically engineered virus has been used successfully to produce an oral vaccine to prevent rabies in wildlife. Research for similar gene splicing in humans continues for possible vectors to carry the replacement genes to their targets. As has been done for animals, there is potential for human – vaccination against certain diseases, or as a targeted delivery capability for therapeutic drugs or cytotoxic effects» [4].

The basis for such statement and prediction of «altering the immunity of animals» comes from the paper «Expression of rabbit IL-4 by recombinant myxoma viruses enhances virulence and overcomes genetic resistance to myxomatosis». In this paper the whole (and detailed) plasmid construction is described (Fig. 2) [7]:

It should be noted that this paper is freely available. This observation is further discussed in a review article M. (Marianne et al.: M. Stanford and Grant McFadden, The 'supervirus'? Lessons from IL-4-expressing poxviruses. Trends in Immunology Vol. 26 No. 6 June 2005):«It is clear from studies in a variety of IL-4-expressing poxviruses that the ectopic expression of this cytokine in the context of virus infection inhibits the formation of antiviral CTL responses. The effect of IL-4 expressed by ECTV on preexisting immunity would suggest that expression of this cytokine modifies the microenvironment, such that protective immune responses cannot properly function. Thus, the effect of ectopic IL-4 expression by poxviruses might include more global effects on the immunopathology, as well as more localized effects on immune cells that bridge the gap between the innate and adaptive immune system, such as the tissue macrophage» [8]. It should be noted that this construction occurred 15 years ago and there is a narrow bridge between the animals and men.

Stealth viruses

«The concept of a stealth virus is a cryptic viral infection that covertly enters human cells (genomes) and then remains dormant for an extended time. However, a signal by an external stimulus could later trigger the virus to activate and cause disease. This mechanism, in fact, occurs fairly commonly in nature. For example, many humans carry herpes virus which can activate to cause oral or genital lesions. Similarly, varicella virus will sometimes reactivate in the form of herpes zoster (shingles) in some people who had chicken pox earlier in life. However, the vast majority of viruses do not cause disease. As a biological weapon, a stealth virus could clandestinely infect the genome of a population. Later, the virus could be activated in the targeted population, or a threat of activation could be used as blackmail. Oncogenes

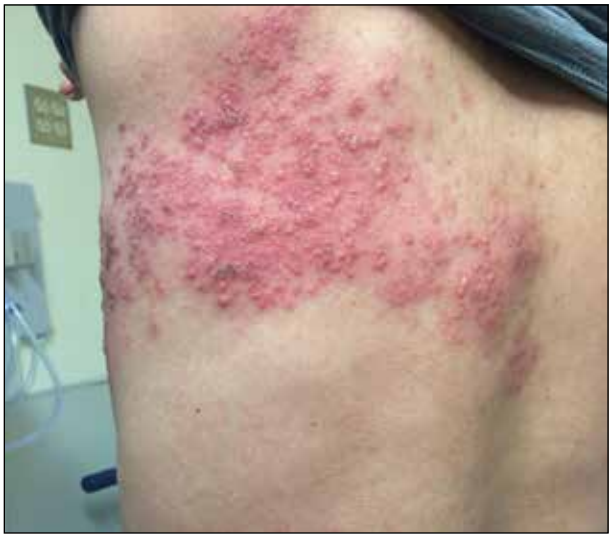

Fig. 3 – ***Herpes zoster. Herpes Zoster (HZ), also known as «shingles,» is a result of the reactivation Varicella Zoster Virus (VZV) that emerges from latency in the sensory dorsal root ganglion. The reactivation causes the spreading of a classic rash of group vesicular lesions in various stages along the unilateral sensory dermatomal distribution over the first 3 days. Ulceration and crusting begin to occur after 3-5 days. 1 The diagnosis is usually made clinically; however PCR testing of skin lesions is also available to differentiate between VZV, HSV1, and HSV2. 2 The incidence of HZ increases with age due to immunosenesacence of cell mediated immunity, with the mean age between 43 and 53 years old. 3 An immunocompromised state, due to factors like human immunodeficiency virus (HIV), medications, and autoimmune disease, also increases the incidence of HZ. 4-6 A routine HIV screening in this patient was negative. He was prescribed oral acyclovir 800 mg, five times per day for five days. (Ehsani-Nia H., Rowe R. Herpes Zoster; https://jetem.org/herpes_zoster/; date: 12.10.2020)***

are segments of DNA that, when switched on, can initiate wild cellular growth and misbehavior – the hallmarks of cancer. Some viruses have segments of DNA that can mimic oncogenes and directly, or perhaps through bioregulators or host genes, cause cancer. These changes may take years for clinical effect, but the concept may still be considered by bioterrorists» [4].

As already mentioned, Herpes zoster, also known as shingles, is caused by the reactivation of the varicella-zoster virus (VZV), the same virus that causes varicella (chickenpox). Primary infection with VZV causes varicella. Once the illness resolves, the virus remains latent in the dorsal root ganglia. VZV can reactive later in a person's life and cause a painful, maculopapular rash called herpes zoster. In some patients particularly in the elderly, the pain continues to persist after the rash heals and develops into

postherpetic neuralgia (PHN), which is the most common complication. PHN causes physical disability, emotional distress and interference with daily activities and sleep [8, 9] (Fig. 3).

HZ also causes neurological sequelae, HZ ophthalmicus (HZO) with eye involvement or disseminated disease. The incidence rate of HZ ranged between 3 and 5/1000 person-years in North America, Europe and Asia-Pacific. The age-specific incidence rates of HZ were similar across countries, with a steep rise after 50 years of age. The incidence rate was about 6–8/1000 person-years at 60 years of age and 8–12/1000 person years at 80 years of age. HZ is a global health burden that is expected to increase as the population ages across the world in the near future. The prevalence of disability in the elderly populations is also increasing(1 -This I do not understand, something is missing). Up to date no specific «activator» of the latent virus is officially known. Its discovery (and use) would disable and ruin whole health care system(s) in particular state(s).

Progressive multifocal leukoencephalopathy (PML) is a rare and often fatal viral disease characterized by progressive damage (-pathy) or inflammation of the white matter (leuko-) of the brain (-encephalo-) at multiple locations (multifocal). It is caused by the John Cuningham virus (JC virus), which is normally present and kept under control by the immune system. The JC virus is harmless except in cases of weakened immune systems. In general, PML has a mortality rate of 30–50% in the first few months, and those who survive can be left with varying degrees of neurological disabilities[4].

JC virus, the etiological agent of progressive multifocal leukoencephalopathy (PML), is the first human polyomavirus described. After asymptomatic primary infection which occurs in childhood, the virus spreads by the hematogenous route from the primary site of infection to secondary sites including kidneys, lymphoid tissues, peripheral blood leukocytes, and brain to establish latent infection. During immunosuppression the virus undergoes molecular rearrangements that allow it to replicate in glial tissues causing PML [910]. Again, the «discovery» of an activator of this stealth virus (and its use) would bring devastating effects on the population of the particular state(s).

Host-swapping diseases

«As previously stated, the vast majority of viruses do not cause disease. In nature, animal viruses tend to have narrow, well-defined host ranges. Unlike bacteria, viruses often infect only one or just a few species. When a virus has a primary reservoir in an animal species, but is transmissible to humans, it is called a zoonotic disease. Animal viruses tend to have a natural animal reservoir where they reside and cause little or no damage. Examples of reservoirs include birds for the West Nile Virus, water fowl for Eastern equine encephalitis and rodents for hantavirus. The bat is thought to be the reservoir for Ebola virus, and the chimpanzee is thought to have been the original reservoir for the HIV virus that causes AIDS. When viruses – jump species they may occasionally cause significant disease. These examples illustrate that manageable infectious agents can be transformed naturally into organisms with markedly increased virulence. When this happens naturally, the process results in an emerging disease. If it were to be induced by man, it would be bioterrorism. In the laboratory of inspired, determined and well-funded bioterrorists, an animal virus may be genetically modified and developed specifically to infect human populations. Emerging diseases could have serious implications for biological warfare or terrorism applications» [4].

Hantaviruses, belonging to the Hantaviridae family, are divided into four genera: Loanvirus, Mobatvirus, Thottimvirus, and Orthohantavirus. More than 50 species of hantaviruses have been reported worldwide[5]. Hantavirus cardiopulmonary syndrome (HCPS) is caused by infection with New World hantaviruses. First described in 1993 in the southwestern United States, HCPS has been documented throughout the Americas Andes hantavirus (ANDV) causes hantavirus cardiopulmonary syndrome and is the only hantavirus for which person-to-person transmission has been proven [10, 11].

The study documented a small but definite risk of nosocomial acquisition of ANDV infection for personnel who care for patients, including handling of bedding and gowns. In the following study the authors conclude: «We characterized the complete genome of an ANDV strain involved in a person-to-person transmission chain by using target-specific whole-genome sequencing. Our study contributed useful data for clarifying properties involved in the unusual transmissibility of ANDV. These data are crucial for optimal management of HPS case-patients and control of future outbreaks of this lethal disease». As stated above: «*In the laboratory of inspired, determined and well-funded bioterrorists, an animal virus may be genetically modified and developed specifically to infect human populations.* What happens if someone would transform this virus (with a case-fatality rate 35–38%) to a more

[4] Progressive multifocal leukoencephalopathy. URL: https://en.wikipedia.org/wiki/Progressive_multifocal_leukoencephalopathy (date: 20.07.2020)

[5] International Committee on Taxonomy of Viruses (ICTV). International Committee on Taxonomy of Viruses (ICTV). 2019 [cited 2019 Oct 10]. URL: https://talk.ictvonline.org/ (date: 20.09.2020)

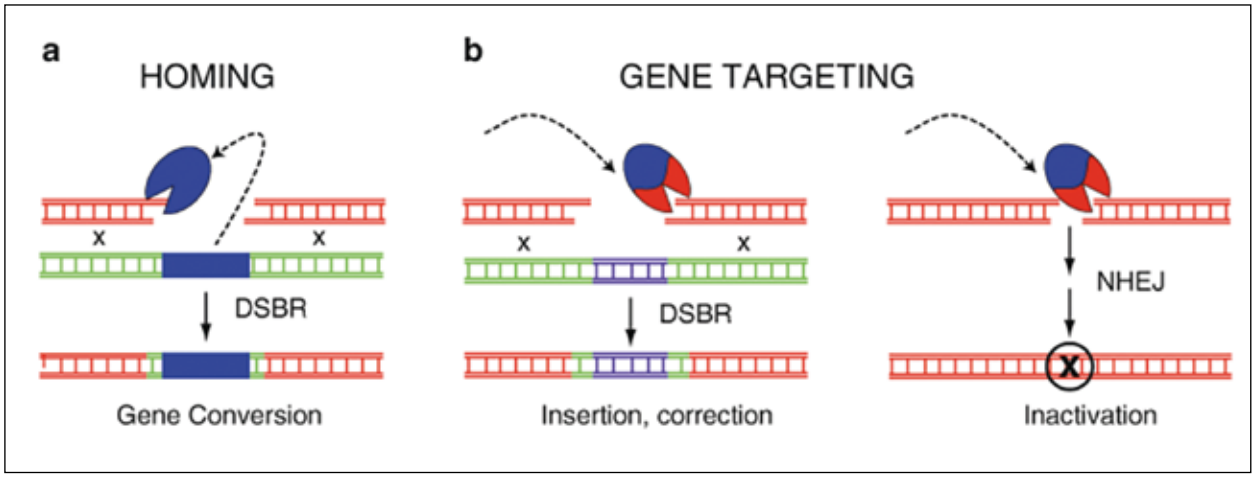

Fig. 4 – *Endonuclease-mediated DNA repair. (a) HE-induced DSB mediates homing. The HEG (blue pacman symbol), often encoded by an intron or intein (blue bar), cleaves an uninterrupted homing site. The DNA ends of the cleaved recipient (red) engage in double-strand-break repair (DSBR), such that the cut allele is repaired by gene conversion with homologous intact DNA. (b) Gene targeting by an exogenous endonuclease. An engineered endonuclease (blue and red) cleaves a desired target, which can be repaired with an allele that inserts or corrects DNA using a homologous template (left), or is repaired by nonhomologous end joining (NHEJ), an error-prone process, that inactivates the cleaved gene [15]*

virulent strain? Moreover, while HCPS is typically associated with New World hantaviruses, the Puumala orthohantavirus in Europe has also caused the syndrome on rare occasions» [11, 12].

Designer diseases

«Our understanding of cellular and molecular biology has advanced nearly to the point where it might be possible to propose the symptoms of a hypothetical disease and then design or create the pathogen to produce the desired disease complex. Designer diseases may work by turning off the immune system, by inducing specific cells to multiply and divide rapidly (like cancer), or possibly by causing the opposite effect, such as initiating programmed cell death (apotosis). This futuristic biotechnology would clearly indicate an order-of-magnitude advancement in offensive biological warfare or terrorism capability. The concepts and mechanisms of the six classes of biological innovations that could be weaponized, as outlined by the JASON Group and discussed above, have some overlap» [4].

These classes were meant to identify a spectrum of conceivable bioterrorist threats based on current or near-future biotechnological capabilities. They were not meant to be all-inclusive or mutually exclusive of possibilities. Another authority on biological warfare, Malcolm Dando asserts that benign microorganisms might be genetically engineered to produce BW toxins, bioregulator compounds, or venoms [12, 13]. Pathogens may also be genetically manipulated to enhance their aerosol or environmental stability, or defeat current identification, detection, and diagnostic capabilities».

Synthetic biology

The term «synthetic biology» can be explained as follows: «Synthetic biology is a newly emerging field that employs the application of engineering principles to biology. Recent advances in synthetic biology have led to the development of several synthetic gene circuits for a wide range of applications including disease diagnostics, disease treatment, production of biomolecules, therapeutics, vaccines, biomaterials, biofuels and fine chemicals. It has allowed us to easily manipulate or build a novel function or upgrade the existing gene function by using a synthetic promoter, ribosome binding sites, genes, transcription terminators, small RNAs and many more in a wide number of organisms and cell types» [13, 14].

There is tremendous amount of literature, facts and results achieved in this field. It would fill couple of articles in this journal. Let us focus only on one aspect – the «preparation» of «synthetic» sterility of mosquito populations. Homing endonucleases are strong drivers of genetic exchange and horizontal transfer of both their own genes and their local genetic environment. This has led to the manipulation and reprogramming of the endonucleases and to their exploitation in genome editing for use as therapeutic agents, for insect vector control and in agriculture [14, 15]. By introducing site-specific DNA breaks that then are repaired, these homing endonucleases (HEs) stimulate the cellular recombination and DNA repair processes to fix the break by simply copying the gene encoding them (the homing endonuclease gene or HEG) and flanking DNA into the broken chromosome (Fig. 4).

Through models of mosquito population genetics and malaria epidemiology combined with currently available HEG transmission data, it was concluded that HEG-based approaches could have a transformational effect on malaria control [15, 16][6]. Nevertheless, one can imagine another (possible) scenario – the use of these methodologies in introducing the sterility in humans. The «vectors» would be exosomes, the gates would be digestive tract or skin (through food or cosmetic articles).

***

Modern biological threats of artificial nature are much more dangerous than those, from which the conventions on the prohibition of weapons of mass destruction are trying to protect us, at least declaratively. The future, which JASON experts wrote about 23 years ago, has already arrived. There are serious reasons to believe that certain biotechnologies have already been used for biological sabotage or they may be used for such purpose in the nearest future. The catastrophic spread of COVID-19 pandemic, uncontrolled by modern medical science, regardless of whether it is artificial or not, clearly shows the limits of human mind and knowledge to resist this and other similar challenges. It is impossible to rely only on «free will» of human mind and on its ability to choose good between the Kantian ideals of good and evil. It is necessary to monitor constantly those new biotechnologies that may have a dual purpose and to improve accordingly the conventional and scientific methods of control over their use.

[6] For more details of the principles of main methodologies for genomic engineering can check the CRISPR and Genomic Engineering. URL: https://www.labome.com/method/CRISPR-and-Genomic-Engineering.html (date: 12.06.2020)

Conflict of interest statement

I am declaring that I prepared the article from sources freely available on the Internet and free available publications, figures and other possible legal sources. I, as a sole author declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest

Peer review information

The article has been peer reviewed by two experts in the respective field. Peer reviews are available from the Editorial Board and from Russian Science Citation Index database.

References

1. Finkbeiner A. The Jasons: the secret history of science's postwar elite. Penguin, 2006.

2. Block S. Living nightmares: biological threats enabled by molecular biology / In: Drell S., Sofaer A., Wilson G. (Eds.), The New Terror: Facing the Threat of Biological and Chemical Weapons. Hoover Institution Press, Stanford, 1999.CA, p. 60).

3. Ryan J.R. Future Directions for Biosecurity, Biosecurity and Bioterrorism, 2016. P. 345–363.

4. Ainscough M.J. Next Generation Bioweapons: The Technology Of Genetic Engineering Applied To Biowarfare And Bioterrorism. Air War College Air University. Maxwell Air Force Base, Alabama. 2004. https://www.researchgate.net/publication/255625820_Next_Generation_Bioweapons_Genetic_Engineering_and_BW

5. Coutarda B., Valleb C., de Lamballerie X. et al. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade // Antiviral Research. 2020. V. 176. 104742. https://doi.org/10.1016/j.antiviral.2020.104742

6. Claas E.C., Osterhaus A.D., Van Beek R. et al. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus // Lancet. 1998. V. 351. P. 472–477. https://doi.org/10.1016/S0140-6736(97)11212-0

7. Kerr P.J., Perkins H D., Inglis B. et al. Expression of rabbit IL-4 by recombinant myxoma viruses enhances virulence and overcomes genetic resistance to myxomatosis // Virology. 2004. V. 324(1). P. 117–128. https://doi.org/10.1016/j.virol.2004.02.031

8. Stanford M., McFadden G. The «supervirus»? Lessons from IL-4-expressing poxviruses // Trends in Immunology. 2005. V.26(6). P. 339–45. https://doi.org/10.1016/j.it.2005.04.001

9. Johnson R.W, Bouhassira D., Kassianos G. et al. The impact of herpes zoster and post-herpetic neuralgia on quality-of-life // BMCMed. 2010. V. 8. P. 37. https://doi.org/10.1186/1741-7015-8-37

10. Pietropaolo V., Prezioso C., Bagnato F., Antonelli G. Cunningham virus: an overview on biology and disease of the etiological agent of the progressive multifocal leukoencephalopathy // New Microbiologica. 2018. V. 41(3). P. 179–186. https://pubmed.ncbi.nlm.nih.gov/29620790/

11. Martinez-Valdebenito C., Calvo M., Vial C. et al. Person-to-Person Household and Nosocomial Transmission of Andes Hantavirus, Southern Chile, 2011 // Emerging Infectious Diseases. 2014. V. 20 (10). P. 1629–1636. http://dx.doi.org/10.3201/eid2010.140353

12. Hong Jiang, Xuyang Zheng, Limei Wang et al.

Hantavirus infection: a global zoonotic challenge // Virol Sin. 2017. V. 32(1). P. 32–43. http://dx.doi.org/10.1007/s12250-016-3899-x

13. Dando M. The New Biological Weapons: Threat Proliferation, and Control. Boulder, CO: Lynne Rienner Publishers, Inc., 2001. https://www.researchgate.net/publication/38141662_The_New_Biological_Weapons_Threat_Proliferation_and_Control

14. Advances in Synthetic Biology /Vijai Singh, Ed: Springer Nature Singapore Pte Ltd., 2020. https://doi.org/10.1007/978-981-15-0081-7

15. Belfort M., Bonocora R.P. Homing Endonucases: From Genetic Anomalies to Programmable Genomic Clippers //Methods Mol. Biol. 2014. V. 1123: 1–26. https://doi.org/10.1007/978-1-62703-968-0_1

16. Deredec A., Godfray H.C., Burt A. Requirements for effective malaria control with homing endonuclease genes // Proc. Natl. Acad. Sci. USA. 2011; V. 108 (43). E874–E880. https://doi.org/10.1073/pnas.1110717108

Author
Centre of Experimental Medicine, SAS, Dubravska cesta 9, 841 04 Bratislava, Slovakia
Faculty of Management Comenius University, Odbojárov 10, 841 04 Bratislava, Slovakia
Ján Lakota. MD, PhD

Contact person: Ján Lakota; jan.lakota@savba.sk

Современные биологические угрозы – там, где прошлые прогнозы встречаются с будущим

Ян Лакота

Центр экспериментальной медицины, Словацкая Академия наук, Дубравская дорога 9, 841 04, Братислава, Словакия
Факультет менеджмента Коменского университета, ул. Бойцы Сопротивления 10, 841 04, Братислава, Словакия

Поступила 12.10.2020 г. Принята к публикации 21.12.2020 г.

Не контролируемое современной медицинской наукой катастрофическое распространение пандемии COVID-19, вне зависимости от того, искусственного она происхождения или нет, наглядно показывает ограниченность возможности человеческого разума и накопленных человеческих знаний противостоять этому и другим подобным вызовам. *Цель работы* – показать опасность биотехнологий двойного назначения при разработке принципиально новых подходов к биологическому поражению людей. В основе методологии исследования – сопоставительный анализ прогнозных оценок развития биотехнологии, сделанных 23 года назад экспертами американской организации JASON (названа по имени предводителя аргонавтов Ясона) и другими специалистами, с ее современным состоянием. Показано, что в основном эти прогнозы подтвердились. Наиболее реальными технологиями в разработке новых средств биологического поражения являются следующие: *бинарные биологические поражающие агенты* – двухкомпонентные системы, которые относительно безопасны в обращении, но становятся смертельно опасными, когда два компонента объединяются при подготовке биологического агента к поражению людей (например, безобидный штамм кишечной палочки и плазмида, превращающая его в вирулентный для человека); *дизайнерские гены* – определенные искусственные последовательности генов встроены в вирусы, меняющие его патогенные свойства, например, сайт для расшепления фурином у SARS-CoV-2, значительно повысившие контагиозность вируса по сравнению с близкородственными видами; *генная терапия* – современная медицинская реальность. Эта технология позволяет врачам восстанавливать или заменять дефектные гены у больного человека, однако она может быть использована для введения в геном здорового человека генных последовательностей, вызывающих патологические состояния; *стелс-вирусы* – вирусы, которые могут быть созданы исследователем для заражения хозяина, но сохраняющие «молчание» до тех пор, пока не будут активированы каким-либо физиологическим или средовым триггером; *болезни с измененным хозяином* – новые агенты, разработанные специально для целей биологической войны на основе возбудителей зоонозных инфекций, ранее не вызывавших болезни у людей; *дизайнерские болезни* – сначала предполагаются определенная патология и симптомы гипотетической болезни,

затем на основе знания биохимических сигнальных путей проектируется или создается патоген, вызывающий такую болезнь. В дополнение к прогнозируемым JASON технологиям двойного назначения, появилась еще одна – *синтетическая биология*. Это междисциплинарная отрасль биологии, в которой применяются инженерные принципы. Она позволяет манипулировать отдельными функциями существующих биологических видов и клеток, и даже создавать новые с помощью синтетических промоторов, сайтов связывания рибосом, генов, терминаторов транскрипции, малых РНК и многих других генетических факторов. Приведены конкретные примеры использования данных технологий для создания новых средств ведения биологической войны и изменения ее характера. Автор считает, что необходимо не только отслеживать новые биотехнологии двойного назначения, но и совершенствовать конвенционные и научные методы контроля за их использованием для ведения биологической войны.

Ключевые слова: *CRISPR; бинарное оружие; биологические угрозы; биологическое оружие; биотехнология; болезнь; вирус; генная инженерия; генная терапия; ДНК; кишечная палочка O104:H4; патогены; синтетическая биология; терроризм.*

Библиографическое описание: *Лакота Ян. Современные биологические угрозы – там, где прошлые прогнозы встречаются с будущим // Вестник войск РХБ защиты. 2020. Т. 4. № 4. С. 421–430. https://doi.org/10.35825/2587-5728-2020-4-4-421-430*

Информация о конфликте интересов

Я заявляю, что статья подготовлена на основании источников, свободно доступных в сети Интернет, а также на основании находящихся в свободном доступе публикаций, иллюстративного материала и других возможных законных источников. Я как единственный автор заявляю, что исследование проводилось без каких-либо коммерческих или финансовых отношений, которые могут быть истолкованы как потенциальный конфликт интересов.

Сведения о рецензировании

Статья прошла открытое рецензирование двумя рецензентами, специалистами в данной области. Рецензии находятся в редакции журнала и в РИНЦе.

Список источников:

Стр. 428–429.

Об авторе

Центр экспериментальной медицины, Словацкая Академия наук, Дубравская дорога 9, 841 04, Братислава, Словакия.

Факультет менеджмента Коменского университета, ул. Бойцы Сопротивления 10, 841 04, Братислава, Словакия.

Ян Лакота, MD, PhD

Контактное лицо: Ян Лакота; jan.lakota@savba.sk