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Mr. Chairman and members of the Committee, thank you for the opportunity to discuss with you the threats presented by biological weapons and biological terrorism. Addressing the issues of engineered biological agents and biological weapons is essential to increasing the understanding of how real the threat is and to determining whether or not it is likely that the United States will have to protect itself from engineered biological weapons in the near future.

In the former Soviet Union, the work to select new strains of virulent pathogens began in the 1970s. As the scientific leader of Biopreparat, the civilian branch of the Soviet Union's offensive biological weapons program, I was responsible for these projects from scientific and financial standpoints. There were a significant number of projects focused on developing various types of new BW, including the ones that involved genetically engineered pathogens. The projects with codenames like "METOL", "FACTOR", "BONFIRE", and "PODLESHIK". These names meant nothing and as I was told they were randomly selected and created by a computer. The work being performed in these programs, however, lead to a grim new reality in weapons development. Among the Soviet Union's areas of interest were new genetically engineered pathogens including antibiotic resistant strains of anthrax, plague, and tularemia; multi-drug resistant glanders and melioidosis; immune-subverting tularemia pathogen, and tularemia and plague pathogens with new virulence factors inserted into them. Of course I am not able to remember the specific details of each project even though I was responsible for all these projects. I had a large number of assistants or as we called them, project creators, who helped me work with principal investigators and institute directors and deputy directors. By 1990, there were approximately 30 project curators coordinating more than 300 projects, some of which involved the development of novel engineered pathogens and weapons, working for me.

One must only look at the Soviet Union's BW program to see that it is possible to develop genetically engineered pathogens. There is no doubt that the probability of developing sophisticated engineered pathogens is more feasible nowadays. It is very difficult to predict what the primary focus would be of a scientific group working on the development of such pathogens. For example, they could focus on the development of antibiotic-resistant pathogens, immune-subverting pathogens, or on pathogens with "added" virulence factors.

Ironically, even though I knew many of Biopreparat's projects during my time as part of the scientific leadership, I learned the details of some of these projects after I moved to the United States and read articles published by my former colleagues between 1992 and 2000.

Interestingly, after 2000-2001 the number of publications in the fields related to biological weapons dropped significantly, then virtually disappeared. Before the disappearance of these types of articles, one could get a significant amount of information about the level genetic engineering research and what could be achieved in the field of biological weapons development. For example, two articles I read described very sophisticated work that focused on the creation of new, genetically engineered pathogens by inserting the human gene, beta endorphin, into *F. tularensis* and a smallpox performed using on non-virulent microorganisms, but anyone with an understanding of microbiology and molecular biology would understand how easily these changes could be transferred to pathogenic strains of the same microorganisms.

In the first of these publications a group of scientists studied how an attenuated strain of *F. tularensis* would produce beta endorphin in experimental animals and examined the changes it could induce in them. Immediately after I started reading the article I realized that the main purpose of this work was to create a genetically engineered pathogen that would produce additional pathogenic effects in humans. I found it interesting how they awkwardly tried to explain the necessity of the work.

The article started with a more or less logical explanation of how the beta endorphin could be a good replacement for morphine and other narcotic painkillers and could be used for the management of pain in people with debilitating diseases. It was logical from the point that the beta endorphin, which is produced by brain cells, is a more powerful painkiller than the existing morphine-like drugs. Another benefit of beta endorphin is that it doesn't cause addiction and could be used for a long period of time without causing any significant harm to the patient. The authors also explained that there were obstacles to this approach. For example, beta endorphin is a peptide, meaning it is subject to enzymatic cleavage by various proteases produced by our body and thus wouldn't have a prolonged effect. For this reason, the authors explained, it was necessary to find a way to keep this substance in the body for as long as possible to ensure a prolonged pain killing effect.

Up to this point, the work was logical but as I continued to read, the logic became hazy, then disappeared altogether. The authors suggested that the best way to keep the beta endorphin in the body for a long period of time was to insert a gene of this substance into a vaccine strain of *F. tularensis*, which wouldn't harm the patient, but while it multiplies it would produce the beta endorphin long period of time. I couldn't understand why they would use even a vaccine strain of a pathogen capable of multiplying in our body. Even using a vaccine strain would mean establishing an infection in the patient and so it made no sense to me why anyone would consider inducing an infection in a person to treat them. Additionally, the authors' explanation of using a pathogen to increase the length of time the endorphin was produced was illogical because the pathogen wouldn't stay in the body for a long time. As soon as the immune system developed specific antibodies against this microbe it would be eliminated from the body and the production of beta-endorphin would stop.

A third problem with the logic of this approach was that this type of treatment could be used just once. As soon as the body developed specific antibodies to the microbe future infusions of this "therapeutic preparation" would be ineffective as the microbe wouldn't be able to multiply in the body.

I thought that I might be missing something and continued to read the article. At the end of the article was a fascinating and revealing account of the results they had obtained. The authors

explained that a few days after injecting the experimental animals with modified *F. tularensis* the animals developed severe muscle rigidity and became catatonic. The real reason for this research was obvious and counter to the humane reasons the authors had given at the beginning of the article.

The second article described the effects of beta endorphin when it was inserted in the Vaccinia virus, which can be used as a model for genetic manipulations of the smallpox virus, *Variola major*. The results were close to the same.

This work was funded by the former Soviet Union and I do not mean to imply that Russia is currently involved in this work. These examples are meant only to show what can be achieved in the field of creating genetically engineered pathogens.

In order to clearly understand what is achievable, let me give you a number of other examples that demonstrate the prevalence and level of sophistication of what is going on in the field of modulating pathogenic microorganisms. I am not saying the work described in these articles has a dual purpose and is being used to develop BW. What I want to say is that there exist many different methods and approaches to developing modified pathogens and that biotechnological advancements provide a large number of new examples each year. The modulation of pathogenic microorganism is not science fiction.

These are some examples:

Article One
Biomed Sci. 1991. All-Union Research Institute of Molecular Biology, Novosibirsk region.

Viral chimeric protein including a determinant of myelin basic protein is capable of inducing allergic encephalomyelitis in guinea pigs.

Shchelkunov SN, Stavitskii SB, Batenko LI, Gashnikov PV, Shchelkunova GA, Kostyrev OA, Sandakhchiev LS.

- A hybrid vaccinia virus expressing a chimeric protein consisting of thymidine kinase and the encephalitogenic determinant, S1, from guinea pig myelin basic protein was constructed. Infection of guinea pigs with the virus resulted in the development of allergic encephalomyelitis.

Article Two
Vopr Virusol. 2000 Nov-Dec;45(6):38-41.

[Immunogenicity of a recombinant strain of vaccinia virus, expressing a Venezuelan equine encephalomyelitis virus structural protein gene in peroral immunization]

Sviatchenko VA, Kiselev NN, Ryzhikov AB, Bulychev LE, Mikriukova TP, Netesov SV.

- Immunogenicity of recombinant vaccinia virus strain (VR26) expressing Venezuelan equine encephalomyelitis (VEE) virus structural protein genes was studied by oral immunization. Sera of animals immunized with VR26 contained antibodies specific to VEE virus, among which antibodies with virus-neutralizing activity were present. Evaluation of the protective efficiency of oral immunization with VR26 demonstrated a high level of animal protection from lethal doses of VEE virus. Rabbits immunized orally were highly resistant (protection index 142.9) to intranasal infection, which is of priority importance for antiVEE vaccine. Comparative analysis of the results of scarification and oral immunization with VR26 indicates that the type of immune response depends on the method of immunization. These results demonstrate good prospects of oral vaccination with recombinant VR26 strain for immunoprophylaxis of VEE.

Article Three
Proc Natl Acad Sci U S A. 1983 Sep;80(17):5364-8.

Construction of live vaccines by using genetically engineered poxviruses: biological activity of recombinant vaccinia virus expressing influenza virus hemagglutinin.

Panicali D, Davis SW, Weinberg RL, Paoletti E.

Recombinant vaccinia viruses containing the cloned hemagglutinin (HA) gene from influenza virus were constructed. The biological activity of these poxvirus vectors was demonstrated both in vitro and in vivo. Expression of HA in cells infected with recombinant vaccinia was detected by using specific anti-HA antiserum and 125I-labeled protein A, showing that HA synthesized under the regulation of vaccinia virus was antigenic. Immunization of rabbits with these recombinant poxviruses resulted in the production of antibodies reactive with authentic influenza HA as detected by radioimmunoassay, by inhibition of HA erythrocyte agglutination, and by neutralization of influenza virus infectivity. The production of antibodies directed against influenza HA suggested that the HA gene expressed in vaccinia is immunogenic. These data indicate the potential of genetically engineered poxviruses for use as generic live vaccine vehicles that have both human and veterinary applications.

Article Four
FEBS Lett. 1993 Mar 15;319(1-2):80-3.

Genes of variola and vaccinia viruses necessary to overcome the host protective mechanisms.

Shchelkunov SN, Blinov VM, Sandakhchiev LS.

Institute of Molecular Biology NPO Vector, Koltsovo, Novosibirsk region, Russian Federation.

Analysis of variola virus nucleotide sequence revealed proteins belonging to several families which provide the virus with the possibility of overcoming the barriers of specific and non-specific host defence against viral infection. The complement-binding proteins, lymphokine-binding proteins, and serine protease inhibitors can be assigned to this type, as can the proteins

providing the orthopoxviruses with resistance to interferon. The revealed differences between the genes (proteins) of variola and vaccinia viruses under study are discussed.

Article Five
Vopr Virusol. 1997 May-Jun;42(3):115-20.

[Immunobiological properties of vp24 protein of Ebola virus expressed by recombinant vaccinia virus]

[Article in Russian]

Chepurnov AA, Ternovoi VA, Dadaeva AA, Dmitriev IP, Sizikova LP, Volchkov VE, Kudoiarova NM, Rudzevich TN, Netesov SV.
Immunological and biochemical parameters were studied in guinea pigs immunized with recombinant vaccinia virus containing full-sized gene of Ebola virus vp24 protein and then infected with virulent strain of Ebola virus. The majority of the studied parameters changed similarly in guinea pigs immunized with recombinant vaccinia virus and control guinea pigs inoculated with vaccinia virus both before and after challenge with Ebola virus. However, in animals immunized with recombinant vaccinia virus producing vp24 some biochemical parameters, the mean life span after challenge with Ebola virus, the level of antibodies to the virus, and the phagocytic activity of neutrophils indicated the development of immunological processes other than in controls, namely, the development of immune response to vp24. Although these processes did not eventually lead to the survival of animals, they prolonged the mean life span and resulted in the production of anti-Ebola antibodies, though the level thereof was low. These data demonstrate that recombinant vaccines against Ebola fever are a promising trend of research

Article Six
Mol Gen Mikrobiol Virusol. 1997(3):24-7.

- **Recombinant vaccinia virus expressing Japanese encephalitis virus protein E]**

  Cheshenko NV, Petrov VS, Protopopova EV, Netesova NA, Konovalova SN, Belavin PA, Loktev VB, Malygin EG.

  Recombinant vaccinia virus expressing protein E of Japanese encephalitis virus has been constructed. Polyclonal antibodies to JE virus reacted with recombinant protein E in immunoblotting. Immunochemical analysis of the recombinant protein E with monoclonal antibodies showed that both group specific and receptor domains of the protein were intact.

Article Sevent
J Virol. 2001 Feb;75(3):1205-10.

Expression of mouse interleukin-4 by a recombinant ectromelia virus suppresses cytolytic lymphocyte responses and overcomes genetic resistance to mousepox.

Jackson RJ, Ramsay AJ, Christensen CD, Beaton S, Hall DF, Ramshaw IA.

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- Genetic resistance to clinical mousepox (ectromelia virus) varies among inbred laboratory mice and is characterized by an effective natural killer (NK) response and the early onset of a strong CD8(+) cytotoxic T-lymphocyte (CTL) response in resistant mice. We have investigated the influence of virus-expressed mouse interleukin-4 (IL-4) on the cell-mediated response during infection. It was observed that expression of IL-4 by a thymidine kinase-positive ectromelia virus suppressed cytolytic responses of NK and CTL and the expression of gamma interferon by the latter. Genetically resistant mice infected with the IL-4-expressing virus developed symptoms of acute mousepox accompanied by high mortality, similar to the disease seen when genetically sensitive mice are infected with the virulent Moscow strain. Strikingly, infection of recently immunized genetically resistant mice with the virus expressing IL-4 also resulted in significant mortality due to fulminant mousepox. These data therefore suggest that virus-encoded IL-4 not only suppresses primary antiviral cell-mediated immune responses but also can inhibit the expression of immune memory responses.

Dear members of the committee.
These examples show the level of sophistication that already has been achieved in the areas of creating genetically engineered pathogenic microorganisms. Unfortunately, these or similar, techniques are already available to countries suspected of being interested in developing biological weapons or that are working on dual-use technologies. However, we need to be cautious before stating that terrorist groups are able to develop sophisticated genetically engineered pathogens. Groups that are not state sponsored do not have the level of scientific sophistication needed to develop such pathogens at this point of time. Of course, that does not mean they will not develop this sophistication in the future or that they would not be able to obtain such strains. Though the threat of terrorist groups developing genetically engineered pathogens may not be immediate, it is important to recognize that it could be a threat in the future. We must diligently monitor the situation and be on the look out for possible changes in the field that could increase the availability of this technology to terrorist groups so that we can be best prepared for possible bioterrorism attacks involving genetically engineered pathogens.