

# Scientific Session of the General Meeting of the RAS Members “The Role of Science in Overcoming Pandemics and Postcrisis Development of Society”

## Plant Viruses: New Opportunities under the Pandemic

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**Abstract**—During the pandemic, an urgent task has become to develop new vaccine platforms that will help fight the infection caused by SARS-CoV-2 and quickly respond to newly emerging pathogens. Plant viruses can make a significant contribution to the solution of this problem. Phytoviruses, having the properties of any viral particles (self-assembly, immunogenicity, nanosize), are safe for humans since plants and mammals have no common infectious agents. As a result of thermal rearrangement of the tobacco mosaic virus, spherical particles of a protein nature have been obtained, which have unique immunostimulation and adsorption properties and can play the role of a universal adjuvant platform to create vaccines. Based on these particles, a scheme for obtaining vaccine preparations is proposed. This technology resembles a toy construction set for children. The basis is spherical particles, on the surface of which there are toy blocks—antigens. The “blocks” can be removed, added, or replaced, and this does not take much time and resources. Based on spherical particles, a polyvalent vaccine candidate against COVID-19 has been created as an adjuvant platform.

**Keywords:** plant viruses, tobacco mosaic virus, adjuvant platform, spherical particles, vaccines, vaccine against SARS-CoV-2

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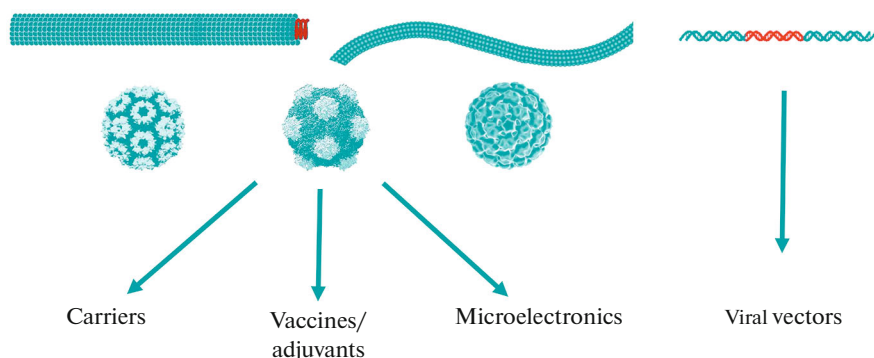
It is clear today that vaccination is the main way to combat the pandemic caused by the SARS-CoV-2 virus. In our country, effective vaccines have been created that are not inferior and that are, perhaps, in some ways superior to foreign ones. During their development, previously obtained and tested platforms were used, which made it possible to release safe vaccine preparations into civilian circulation in record time.

The pandemic will continue. Moreover, virologists are sure that we will encounter other zoonotic infections; viruses will repeatedly overcome the interspecies barrier, and the human population will fight new infectious agents. In this regard, of course, it is topical to develop new platforms and panels of platforms for creating vaccines, which will allow us to respond quickly to challenges and fight newly emerging infections [1]. Plant viruses can make a significant contribution to the creation of vaccine platforms. Recall that about 40 years ago Academician J.G. Atabekov created a scientific school of molecular biology of plant

viruses in our country. It was internationally recognized, and brilliant scientific results were obtained in plant virus basic research. Applied work related to the fight against viral infections, which cause significant damage to agriculture, was also developing successfully.

Over the past 10–15 years, the situation in phytovirology has changed dramatically. The results of research in the field of molecular biology of plant viruses have become of great importance not only for basic science and agriculture but also for the creation of new biotechnologies. Plant viruses, having the properties of all viral particles (self-assembly, immunogenicity, nanosize), are safe for humans since plants and mammals have no common infectious agents [2]. Obtaining purified preparations of phytoviruses is an extremely low-cost process since it does not require sophisticated equipment, sterility, culture media, etc. In addition, methods for isolating plant viruses from plant material have long been well developed, which makes it possible to obtain completely purified preparations that do not contain any impurities. In this context, plant viruses are studied and used in perfectly different areas of biotechnology. They are used as carriers for functionally active molecules; they become the basis for vaccines and can be considered as adjuvants;

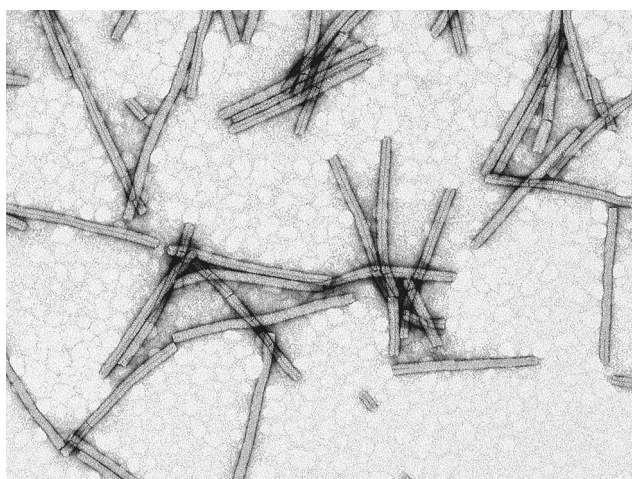
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**Fig. 1.** Main directions of applied research of plant viruses in the field of biotechnology and medicine. Schematic representations of the tobacco mosaic virus, potato mosaic virus X, bonfire mosaic virus (PDB: 1JS9), cowpea mosaic virus (PDB: 1NY7), and tomato bush virus (PDB: 2TBV) are shown.

microelectronic devices are made on their basis; and they are used as viral vectors for the expression of target proteins in plants (Fig. 1) [3].

Note that thanks to Atabekov's scientific school, we are at the forefront of all these developments, and the research that is being carried out in our country is pioneering. Of course, one of the objects of research has been the tobacco mosaic virus (TMV)—a favorite object of phytovirologists, the first virus that was discovered by D.I. Ivanovskii in 1892. Figure 2 shows a classic image of the TMV, obtained using the method of transmission electron microscopy. We have shown that if the TMV is heated for several minutes at 94°C, a rod-shaped virion with a helical structure 18 nm in diameter and 300 nm long turns into spherical particles (SPs), the size of which can be controlled depending on the initial concentration of the viral preparation from 50 to 1000 nm or more [4]. Figure 3 shows SPs formed when heating the tobacco mosaic virus to



**Fig. 2.** Micrograph of tobacco mosaic virus. The image was obtained in the Department of Virology, Faculty of Biology, Moscow State University, on a Jem-1011 transmission electron microscope (Jeol, Japan).

94°C for 10 s with an initial concentration of 1 mg/mL, obtained by scanning electron microscopy. During the thermal rearrangement of the virion into a spherical particle, the TMV genome is released from the protein coat and remains in solution. Therefore, SPs consist only of the virus coat protein. Analysis of ultrathin sections of the spherical particles made it possible to establish that they were homogeneous and did not have cavities inside [5]. Their density is 1.43 mg/mL, which differs significantly from the density of TMV virions (1.31 mg/mL) [6]. That is, during thermal rearrangement, the TMV envelope protein undergoes serious conformational changes, which leads to the formation of denser spherical particles than TMV virions. SPs have a number of amazing properties. One of the most important is the ability to serve as an effective adjuvant (immunostimulant) [7].

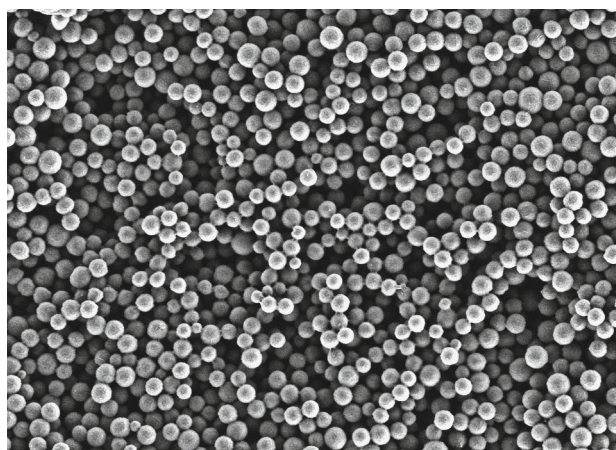
Adjuvants are actively used in modern medical practice to enhance the body's immune response during vaccination against human and animal infectious agents. Various classes of compounds exhibit adjuvant activity, such as bacteria, mineral salts, emulsions, microparticles, small molecules, saponins, and liposomes. However, only some of them have been permitted for use in medical practice [8]. The main problems of adjuvants currently used in the production of vaccines are their low efficiency, the ability to affect the vital activity of the body negatively, and the difficulty in removing adjuvant drugs from the body after vaccination. In this context, the creation of a new effective, biodegradable, and cheap adjuvant is an extremely urgent task of modern molecular medicine, vaccinology, and virology. Plant viruses may be promising immunostimulants with such properties. Some features of the structural organization and size of phytoviruses allow them to stimulate the immune system of mammals effectively [9].

We have carried out the first comparative study of the adjuvant properties of plant viruses of various shapes and sizes, containing different genetic material. Tobacco mosaic virus (rod-shaped) and potato virus X

(filamentous) were used in the experiment. Both viruses contain a genome in the form of RNA. Their immunostimulatory properties were compared with viruses with an icosahedral symmetry type—the bean soft mosaic virus with an RNA genome and the cauliflower mosaic virus, the genetic material of which is presented in the form of DNA [10]. The same experiments also involved spherical particles obtained from TMV. Ovalbumin and lysozyme were used as model antigens. It turned out that at least two out of four viruses and SPs can work as effective adjuvants, that is, actively stimulate the production of immunoglobulins G (IgG) to target proteins compared to IgG titers in laboratory animals immunized only with model antigens [10]. Note that SPs in comparative experiments were significantly superior in their immunostimulatory properties to aluminum compounds, which are now actively used in existing vaccines, and were also comparable with Freund's adjuvant, which is permitted for use only in laboratory conditions [7, 11]. The results obtained allow us to state that plant viruses and their structurally modified virions (spherical particles) have significant immunostimulatory properties and can potentially become safe, effective, and cheap adjuvants.

In addition to high immunostimulatory performance, spherical particles have a number of other features that allow us to consider them as a promising platform for creating vaccine preparations. SPs are extremely stable; they can be reheated to 94–98°C, frozen in the range from –18 to –70°C, and stored at 4°C and even at room temperature for more than six months. Nothing happens to them: their shape, size, and aggregation state do not change. SPs are completely biosafe. A large series of experiments demonstrated no toxicity (acute, chronic, reproductive, or immune) in various types of laboratory animals [12, 13]. It was mentioned above that plants and mammals do not have common pathogens, but spherical particles do not contain a nucleic acid—the virus genome; they consist only of an envelope protein and therefore do not pose any danger to mammals, primarily to humans [4]. Unlike TMV virions, SPs are biodegradable. It has been shown that when an SPs preparation is treated in the presence of structurally unmodified TMV virions with proteinase K, only TMV particles remain within view when analyzed by transmission electron microscopy, while spherical particles are completely hydrolyzed [14].

The most important feature of spherical particles is their unique adsorption properties. Proteins of any size and amino acid composition can be adsorbed on their surface due to hydrophobic bonds and electrostatic interaction. Using immunofluorescence microscopy, we have demonstrated in a number of studies that the potato virus X envelope protein, hemagglutinin polypeptide, epitope M2 of influenza virus protein, tetraepitope A of the E1 glycoprotein of the rubella virus, and the recombinant plum pox virus antigen can land



**Fig. 3.** Spherical particles obtained by thermal rearrangement of TMV. The image was obtained at the Department of Virology, Faculty of Biology, Moscow State University, using a Merlin scanning electron microscope (Carl Zeiss, Germany).

on the SPs surface. All adsorbed proteins retained their antigenic specificity and were available for interaction with the corresponding antibodies as part of complexes with spherical particles [7]. In addition to individual proteins, whole virions of small nonenveloped icosahedral viruses (26–50 nm in diameter) can be adsorbed on the SPs surface, the viruses being infectious agents of humans, farm animals, and plants [15]. Moreover, it is to these target proteins or full-length virions that an effective immune response is developed when using “SPs–target protein (virion)” complexes as vaccine candidates.

After the first experiments to assess the immunogenicity of such complexes in laboratory animals, it became clear that the formation of a complex with SPs not only allows the production of antibodies to the target antigen but also increases the efficiency of the immune response compared to immunization with the same antigen without SPs. That is, spherical particles not only act as a platform for the presentation of target antigen proteins but also have the properties of an effective adjuvant. This means that SPs can enhance the production of antibodies to protein antigens, regardless of their structure, size, and origin. Importantly, in all studies using a significant number of various antigens, the immune response to them in the complex with SPs was higher compared to the immune response to the corresponding individual antigens. We suggest that the gradual dissociation of antigens from the surface of SPs, which probably act as a depot for target proteins, can increase the stimulation of the immune response [15].

In addition, work was done to compare the number of antibodies produced against the antigen and SPs during immunization with SPs–antigen compositions. It turned out that the immune response to the



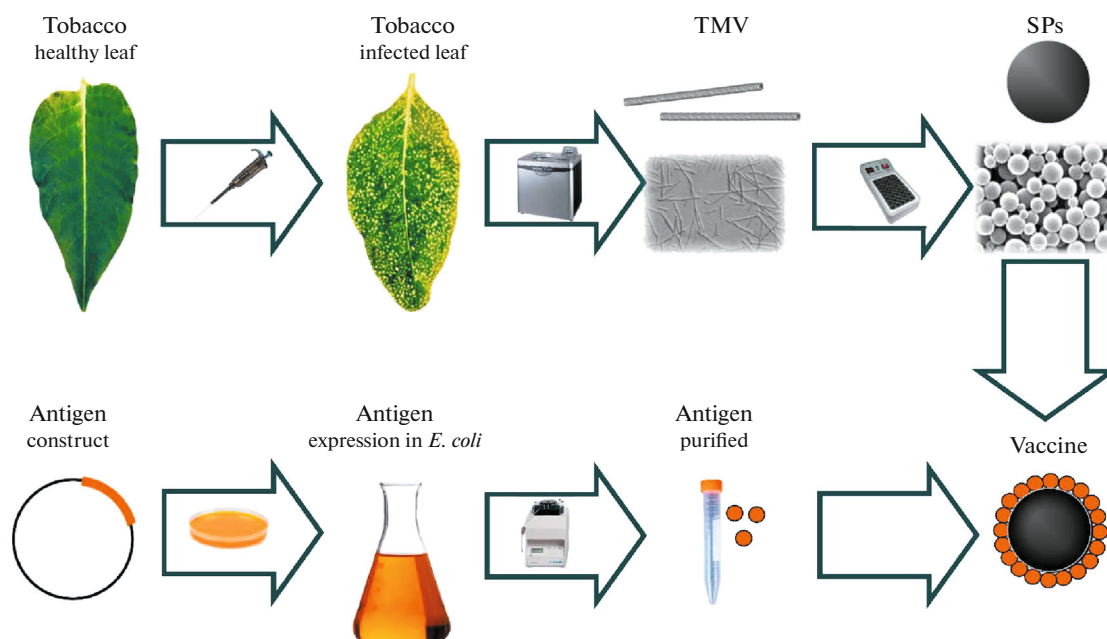


Fig. 4. Scheme for obtaining a vaccine candidate based on a structurally modified tobacco mosaic virus.

target antigen is 6–40 times higher depending on the type of the antigen, that is, spherical particles activate the immune response to the antigen that is in the composition with them and not to their own protein [10, 11]. Since SPs are an adjuvant of a protein nature, the ability to induce the production of a higher antibody titer to the target antigen, rather than to the adjuvant, is an important property that makes them particularly attractive as a universal adjuvant platform.

Based on the properties of spherical particles, we created a scheme for obtaining a vaccine candidate (Fig. 4). The proposed approach is completely universal. Using this technology, it is possible to create a vaccine against almost any human pathogen of a viral or bacterial nature. The scheme for obtaining a vaccine candidate is quite simple. Specially grown leaves of cultivated tobacco (*Nicotiana tabacum*) are mechanically infected with TMV; after incubation for approximately three weeks, the leaves are harvested and frozen. A purified tobacco mosaic virus preparation is isolated from the infected plant material by differential centrifugation. To obtain SPs, the initial virus preparation with a given concentration is incubated in a thermostat at 94°C for 5 min. As a result, spherical particles of a controlled size are formed. In parallel, with the help of genetic engineering approaches, a genetic construct of a recombinant protein, the target antigen, is created. The expression of the genetic construct can occur in cells of any nature. Figure 4 shows the expression of antigens in *Escherichia coli* bacterial cells. During incubation, due to the unique adsorption properties of spherical particles, antigens are located on their surface. As a result, we get a prac-

tically ready vaccine candidate. In addition, we showed the possibility of simultaneous landing of three or more antigens on the SP surface, which indicates the prospect of creating polyvalent vaccines based on spherical particles [7, 16]. The proposed technology can be compared with a toy construction set for children. There is a base, spherical particles, on the surface of which there are “blocks”—antigens. They can be removed, added, or replaced without spending a lot of time or resources.

Based on the structurally modified tobacco mosaic virus (SPs), we created several prototypes of vaccine candidates against various infections of both viral and bacterial nature (see Table 1), as well as samples of candidate vaccines against human rotavirus, anthrax, avian influenza, etc. A rubella vaccine candidate based on spherical particles for women of reproductive age, children, and people with immunodeficiency has already successfully passed preclinical trials. Using immunoelectron microscopy and polyclonal serum obtained against rubella virions, as well as gold particles conjugated with secondary antibodies as a marker, we saw the architecture of the SPs—rubella antigen complex. It turned out that in this case, the rubella virus virion is practically imitated. The antigen regularly covers the surface of the SPs in the same way as the surface glycoprotein E (the main antigen of the rubella virus) is located on the surface of the virion [11]. Only an SPs-based vaccine candidate is absolutely safe and nonreactogenic compared to an attenuated vaccine containing rubella virus particles; in addition, the price of the attenuated vaccine is incomparably higher.

**Table 1.** Samples of candidate vaccines based on the structurally modified tobacco mosaic virus (SPs)

Infection	Antigen	Reference
Rubella	A4 epitope	[11]
Bird flu	HA and M2e proteins	[16]
Rotavirus infection	RV14 epitope of VP6 protein	[17]
Hantavirus infection	Inactivated Puumala virus	[18]
COVID-19	Antigens Co1, CoF, and PE	[19]
Anthrax	PA protein	[20]
Rabies	Inactivated rabies virus	[21]

In the context of the pandemic, relying our experience in creating vaccine candidates, we began to work on the creation of a vaccine against COVID-19, caused by the SARS-CoV-2 coronavirus. Based on spherical particles, a polyvalent vaccine preparation was created as an adjuvant platform: three recombinant antigens, including the RBD domain and conserved epitopes from the S1 and S2 domains of the S protein, were simultaneously adsorbed on SPs. One of these antigens is a conserved amino acid sequence common to a number of betacoronaviruses. This approach may make it possible to receive a vaccine preparation in advance to combat coronaviruses that may cross the interspecies barrier in the future. Immunofluorescence microscopy has shown that all three antigens are adsorbed on the surface of spherical particles [19]. All antigens on the surface of SPs can easily be replaced by others that differ in the amino acid composition. One can also change the number of antigens on the surface of the SPs. Such adjustments can be made to the vaccine preparation in a short time, depending on the epidemic situation: if new strains of SARS-CoV-2 emerge, or if other betacoronaviruses cross the interspecies barrier.

At present, the high immunogenicity of such complexes and the virus-neutralizing activity of the sera of animals immunized with a vaccine candidate have been shown. Experiments were carried out on mice and hamsters; preliminary results on the biosafety of the drug were obtained on the same animals [19]. Preparations are underway to study the vaccine in primates. The World Health Organization has included the vaccine candidate against SARS-CoV-2 based on plant viruses in the list of promising approaches [22].

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#### CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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