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Immunogenicity of the DNA/Protein Combined Vaccine against COVID-19

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During the COVID-19 pandemic, the development of prophylactic vaccines, including those based on new platforms, became highly relevant. One such platform is the creation of vaccines combining DNA and protein components in one construct. For the creation of DNA vaccine, we chose the full-length spike protein (S) of the SARS-CoV-2 virus and used the recombinant receptor-binding domain (RBD) of the S protein produced in CHO-K1 cells as a protein component. The immunogenicity of the developed combined vaccine and its individual components was compared and the contribution of each component to the induction of the immune response was analyzed. The combined DNA/protein vaccine possesses the advantages of both underlying approaches and is capable of inducing both humoral (similar to subunit vaccines) and cellular (similar to DNA vaccines) immunity.

Key Words: *SARS-CoV-2; DNA vaccines; subunit vaccines; DNA/protein vaccines; immune response*

The global response to the acute respiratory syndrome pandemic (SARS-CoV-2) was unprecedented in public health history. The development of anti-COVID-19 vaccines has involved both traditional and innovative approaches, resulting in the development of nucleic acid-based vaccines (mRNA, DNA), vector, and recombinant protein vaccines [1]. DNA vaccines are considered to be good inducers of cellular immunity [2], while subunit vaccines stimulate the production of specific and neutralizing antibodies [3]. Various reviews discuss the necessity of stimulation of both types of immune response for protection against infection caused by SARS-CoV-2 [4,5].

We obtained a vaccine construct combining two approaches: a DNA vaccine and a recombinant protein. The construct is an artificial self-assembled particle with a DNA vaccine encoding the S protein of the SARS-CoV-2 virus as its core. The second component of the vaccine is a polycationic polyglycine-spermidine (PGS) conjugate with the receptor-binding domain (RBD) of protein S, which binds electrostatically to the negatively charged vaccine DNA to form a particle coat.

The aim of this work is to study the immunogenic properties of a combined DNA/protein vaccine called CCV-S (Combined Coronavirus Vaccine).

MATERIALS AND METHODS

The sequence of the gene encoding the full-length protein S of the SARS-CoV-2 virus Wuhan (GenBank MN908947) was used to construct the DNA vaccine. This sequence was optimized for expression in mammalian cells, contained a tissue plasminogen activator

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signal peptide, Tpa (MDAMKRGLCCVLLLCGAVFVSA), was synthesized as part of the pVAX plasmid vector in DNK-Sintez Company, and was named pVAXs. Plasmid production and isolation were performed as described in [6]. The expression of protein S gene was studied using HEK293 cells transfected with pVAXs plasmid. Cell lysates and culture medium in which transfected cells grew were tested for the presence of S protein using immunoblotting.

The S and RBD proteins were prepared as previously described [7]. RBD protein was conjugated to PGS [8]. PGS is a polycationic carrier used to deliver DNA vaccines [6,9,10]. PGS and PGS-RBD conjugates were used to generate two types of particles (Fig. 1, *a*). For the formation of CCV-S particles, DNA was mixed with the PGS-RBD conjugate at a ratio of 50 μg of protein per 100 μg of DNA, incubated for 5 min at room temperature, then PGS was added in 10-fold excess and incubated for 1 h at 4°C. To form pVAXs-PGS particles, DNA was mixed with PGS conjugate in the mass ratio 1:10 and incubated for 1 h at 4°C. The efficiency of complex formation was assessed by changes in electrophoretic mobility of DNA in 1% agarose gel, as well as by gel-filtration on a Sepharose CL-6B column [8]. Samples for gel filtration were applied to the column in equimolar amounts of nucleotide material.

Immunogenicity of created constructs was tested on female BALB/c mice (n=32) weighing 16-18 g. All animal experiments were performed in accordance with the principles of humanity; the study protocols were approved by the Bioethics Committee of State Research Center of Virology and Biotechnology "VECTOR" (authorization number: SRC VB "VECTOR"/10.09.2020). Mice were divided into 4 groups (8 animals each): 3 experimental and 1 control. The experimental groups were immunized with either combined vaccine containing 100 μg DNA and 50 μg protein (CCV-S group), or with 100 μg of pVAXs plasmid encapsulated in a PGS sheath (pVAXs-PGS group), or with 50 μg of RBD protein (RBD group). Control (intact) mice were not immunized and served as the negative control. The mice were immunized intramuscularly into the thigh of the hind limb twice with an interval of 3 weeks. In 10 days after the second immunization, the blood and spleens were taken from the animals for evaluation of the immune response. The materials were prepared for analysis and immunological tests (ELISA, virus-neutralization, ELISpot, and intracellular cytokine staining, ICS) as described previously [8].

The results were processed using the nonparametric Mann–Whitney test (GraphPad Prism 6.0 software). The differences were considered significant at $p<0.05$.

RESULTS

DNA construct pVAXs was tested for its ability to ensure *S* gene expression in eukaryotic cells by immunoblotting with monoclonal antibodies to protein S. Protein S was detected only in the lysate of pVAXs transfected cells and was not detected in the culture medium, which suggests that the protein product of gene *S* is not secreted from the cell in quantities sufficient for detection. These results suggest that pVAXs provides expression of the gene encoding S protein in eukaryotic cells.

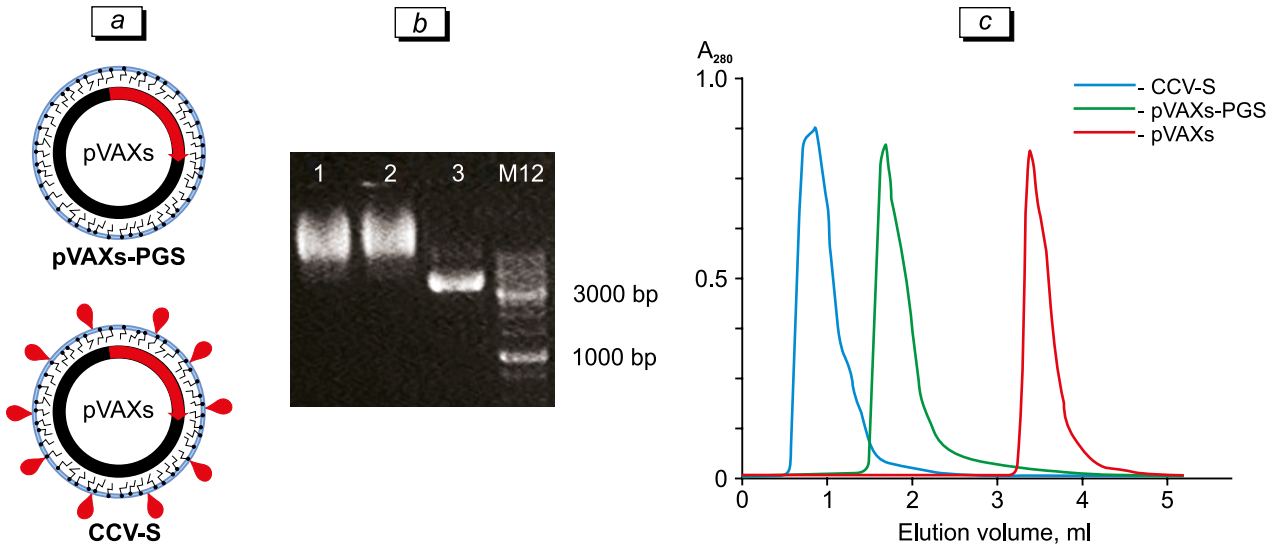

Fig. 1. Analysis of the formation of CCV-S and pVAXs-PGS complexes. *a*) Schematic representation of CCV-S and pVAXs-PGS particles (made using biorender.com); *b*) electrophoretic analysis of particle formation in 1% agarose (1 — pVAXs-PGS, 2 — CCV-S, 3 — pVAXs); *c*) gel filtration of CCV-S, pVAXs-PGS particles and pVAXs plasmids on a Sepharose CL-6B column (chromatographic profile).

The second vaccine component, RBD protein, was purified by standard chromatography (purity >98%). The recombinant RBD was covalently attached to a polycationic carrier, the PGS conjugate. This carrier has been tested in various immunogenicity studies of DNA and combined vaccines and has proven to be a safe and effective vehicle for DNA vaccines [6,9,10].

The combined DNA/protein vaccine (CCV-S) was obtained by self-assembly of pVAXs and PGS-RBD. Particles containing pVAXs plasmid inside and their envelope consisting of PGS (without RBD protein) served as a control (Fig. 1, *a*). Particle formation was assessed by the shift in their electrophoretic mobility in agarose gel: encapsulated plasmids lost mobility in the electric field (Fig. 1, *b*). The particle size measured by gel filtration was ⩾50 nm (Fig. 1, *c*).

The immunogenicity of CCV-S was assessed in a BALB/c mouse model, using pVAXs-PGS constructs and RBD protein as controls. The anti-RBD antibody titer in the group immunized with the combined vaccine was, on average, 1:105,300, *i.e.* higher by 110 times than in the group immunized with DNA

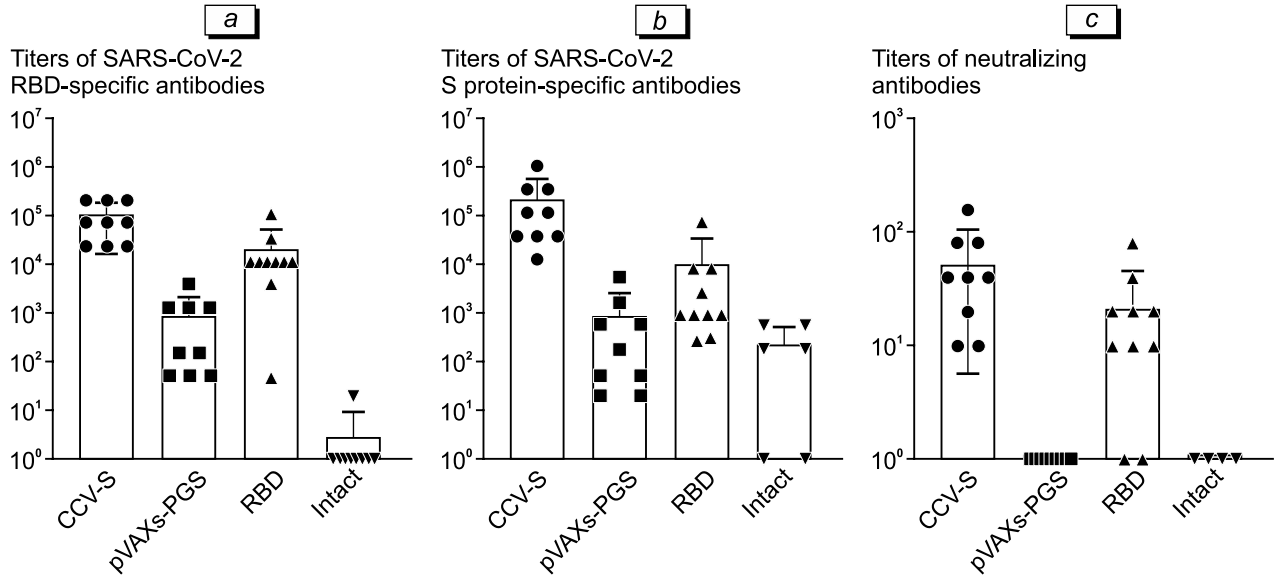

Fig. 2. Humoral immune response in BALB/c mice immunized with CCV-S, pVAXs-PGS constructs, and RBD protein. *a*) Titers of specific IgG to RBD of SARS-CoV-2; *b*) titers of specific IgG to S protein of SARS-CoV-2; *c*) titers of virus-neutralizing antibodies. Viral neutralizing activity was determined using the strain SARS-CoV-2 nCoV/Victoria/1/2020. The data are presented as the mean of reverse titers.

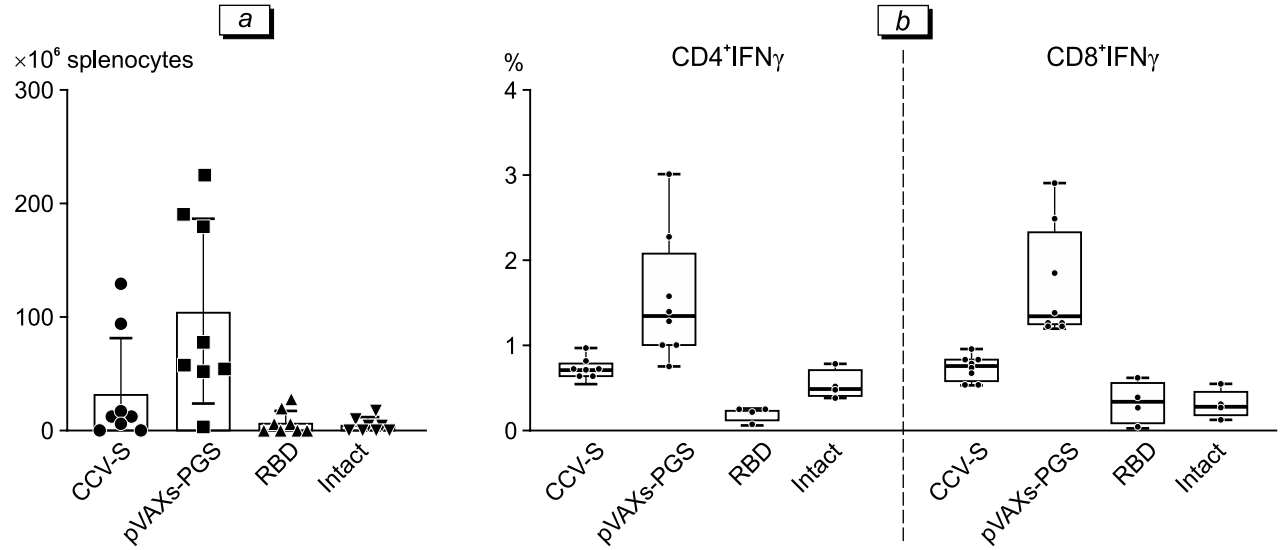

Fig. 3. Cellular immune response in BALB/c mice. *a*) Number of cells producing IFNγ (according to ELISpot), *b*) proportion of SARS-CoV-2-specific IFNγ-producing $CD4^+$ and $CD8^+$ T cells (according to ICS and flow cytometry).

alone (pVAXs-PGS) and by 5 times than in the group immunized with RBD protein ($p<0.01$) (Fig. 2, *a*). Anti-S antibody titer in the CCV-S immunized group was 1:220,000, which was 186-fold higher than in the group immunized with pVAXs-PGS and 23-fold higher than in the group immunized with RBD protein ($p<0.01$) (Fig. 2, *b*). Strengthened immune response to combined vaccine administration in comparison with administration of DNA or protein can indicate more effective binding of CCV particles by antigen-presenting cells as well as stimulation of the T-helper response.

Plasmid pVAXs coated with PGS (without RBD) induced low antibody titers in animals. This fact correlated with the results of immunoblotting, which showed that protein S was not secreted by eukaryotic cells, but remained in cells, which led to a weak humoral response.

An important criterion for vaccine efficacy is the ability to induce antibodies that can neutralize live virus. Only sera from mice immunized with the combined vaccine or protein demonstrated the ability to neutralize the nCoV/Victoria/1/2020 strain of SARS-CoV-2 virus on *in vitro* cell culture in the virus- neutralization reaction (Fig. 2, *c*).

ELISpot (Fig. 3, *a*) and ICS (Fig. 3, *b*) assessed the ability of splenocytes to produce IFNγ in response to stimulation with a pool of peptides from protein S. The highest rates of cellular immunity (both $CD4^+$ and $CD8^+$) were achieved in the group immunized with pVAXs-PGS DNA vaccine. Administration of the combined vaccine also resulted in the formation of cellular immunity in the animals, in contrast to the administration of RBD protein alone (Fig. 3). Similar results were obtained by simultaneous immunization of rhesus macaques with DNA vaccine and SARS-CoV-2 spike protein [11].

Thus, the combined DNA/protein vaccine CCV-S has the ability to induce both high titers of S- and RBD-specific and virus-neutralizing antibodies as well as a cellular $CD4^+$ and $CD8^+$ immune response. CCV-S is a promising candidate vaccine against COVID-19 and the combined vaccine platform will be useful for vaccine design against various diseases.

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