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(71) Applicant: **FEDERAL STATE BUDGETARY INSTITUTION "NATIONAL RESEARCH CENTRE FOR EPIDEMIOLOGY AND MICROBIOLOGY NAMED AFTER THE HONORARY ACADEMICIAN N.F. GAMALEYA" OF THE MINISTRY OF HEALTH OF THE RUSSIAN FEDERATION** [RU/RU]; ul. Gamalei, 18, Moscow, 123098 (RU).

(72) Inventors: **ZUBKOVA, Olga Vadimovna**; ul. Kudryavtseva, 12, kv. 147, g. Khimki, 141401 (RU). **OZHAROVSKAIA, Tatiana Andreevna**; ul. Grabnna, 4, kv. 27, g. Korolev, 141070 (RU). **DOLZHIKOVA, Inna**

Vadimovna; ul. Pushkina, 6, kv. 79, Moskovskaya oblast, g. Lobnya, 141732 (RU). **POPOVA, Olga**; ul. R. Zorge, 47/1, kv. 98, g. Ufa, 450059 (RU). **SHCHEBLIAKOV, Dmitrii Viktorovich**; ul. Mitinskaya, 28, k. 5, kv. 365, Moscow, 125430 (RU). **GROUSOVA, Daria Mikhailovna**; Zadonskiy proezd, 32/2, kv. 25, Moscow, 115682 (RU). **DZHARULLAEVA, Alina Shahmirovna**; Volgogradskiy pr-t, 17, kv. 242, Moscow, 117623 (RU). **TUKHVATULIN, Amir Ildarovich**; Osenniy b-r, 5, k. 3, kv. 945, Moscow, 121609 (RU). **TUKHVATULINA, Natalia Mikhailovna**; Osenniy b-r, 5, k. 3, kv. 945, Moscow, 121609 (RU). **SHCHERBININ, Dmitrii Nikolaevich**; ul. Panfilova, 23, krasnogorskiy r-n, Moskovskaya oblast, p. Nakhabino, 143432 (RU). **ESMAGAMBETOV, Ilias Bulatovich**; ul. Moskovskaya, 5, kv. 22, g. Dmitrov, 141800 (RU). **TOKARSKAYA, Elizaveta Alexandrovna**; ul. Dmitriya Ulyanova, 4, korp. 2, kv. 189, Moscow, 119333 (RU). **BOTIKOV, Andrei Gennadevich**; ul. Rogova, 4, kv. 24, Moscow, 123098 (RU). **BORISEVICH, Sergey Vladimirovich**; prospect Krasnoy Armii, Moskovskaya oblast, g. Sergiyev Posad, 141303 (RU). **NARODITSKY, Boris Savellievich**; ul. Aviatsionnaya, 63, kv. 19, Moscow, 123182 (RU). **LOGUNOV, Denis Yuryevich**; ul. Gamalei, 10, kv. 37, Moscow, 123098 (RU). **GINTSBURG, Alek-**

(54) Title: IMMUNOBIOLOGICAL AGENT FOR INDUCING SPECIFIC IMMUNITY AGAINST SEVERE ACUTE RESPIRATORY SYNDROME VIRUS SARS-COV-2

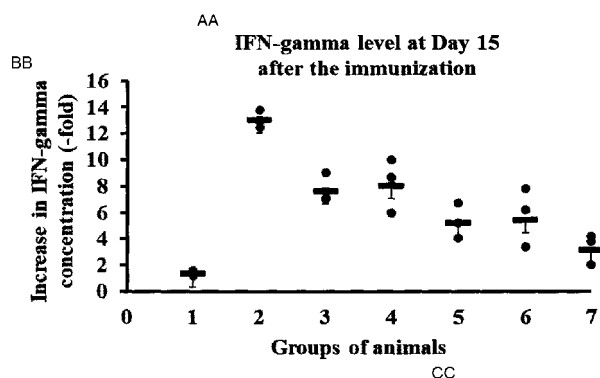


FIG. 5

(57) Abstract: The invention relates to biotechnology, immunology and virology and, in particular, to an immunobiological agent for the prevention of diseases caused by severe acute respiratory syndrome virus SARS-CoV-2. Also, a method of inducing specific immunity to the SARS-CoV-2 virus is disclosed, comprising the administration to mammals of one or more immunobiological agents for the prevention of diseases caused by severe acute respiratory syndrome virus SARS-CoV-2. The invention facilitates an effective induction of the immune response to the SARS-CoV-2 virus.

sandr Leonidovich; ul. Bolshaya Cherkizovskaya, 5, korp. 8, kv. 41, Moscow, 107061 (RU).

(74) **Agent: SIMAKINA, Olga Vasilevna**; ul. Izumrudnaia, 18, kv. 32 (ap. 505A), Moscow, 129346 (RU).

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IMMUNOBIOLOGICAL AGENT FOR INDUCING SPECIFIC IMMUNITY AGAINST
SEVERE ACUTE RESPIRATORY SYNDROME VIRUS SARS-COV-2

Field of the Invention

The invention relates to biotechnology, immunology and virology. The claimed agent can be used for the prevention of diseases caused by severe acute respiratory syndrome virus SARS-CoV-2.

Background of the Invention

SARS-CoV-2 is a new strain of the coronavirus isolated at the end of 2019 in Wuhan (China) which spread around the world within several months. In January 2020, the World Health Organization declared the SARS-CoV-2-related outbreak to be a public health emergency of international concern and in March described the spread of the disease as a pandemic. At the beginning of April 2020, over 1 million cases of illness were confirmed and 60 thousand people died.

The disease caused by SARS-CoV-2 has been given a specific name: COVID-19. It is a potentially severe acute respiratory infection with varying clinical course from mild to severe cases that can cause such complications as pneumonia, acute respiratory distress syndrome, acute respiratory failure, acute heart failure, acute kidney injury, septic shock, cardiomyopathy, etc.

SARS-CoV-2 is spread by human-to-human transmission through an airborne route or direct contact. The basic reproduction number (R_0) of SARS-CoV-2, i.e. the number of people who will catch the disease from a single person, according to different publications ranges from 2.68 (Wu JT, Leung K, Leung GM. Nowcasting and forecasting the potential domestic and international spread of the 2019-nCoV outbreak originating in Wuhan, China: a modelling study. Lancet. 2020) to 6.6 (Sanche S, Lin YT, Xu C, Romero-Severson E, Hengartner N, Ke R. The Novel Coronavirus, 2019-nCoV, is Highly Contagious and More Infectious Than Initially Estimated. medRxiv. 2020) and the median incubation period is 5.2 days (Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y. et al. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. N Engl J Med. 2020).

Phylogenetic analysis of strains isolated from patients with COVID-19 demonstrated that the most closely related to SARS-CoV-2 viruses were found in bats (Zhou P. et al. A

pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020; 579: 270-273). Also, there is an assumption that other mammal species might serve as “intermediate hosts” in which SARS-CoV-2 could acquire some or all mutations needed for its effective transmission to human (Zhang YZ, Holmes EC. A Genomic Perspective on the Origin and Emergence of SARS-CoV-2. *Cell*. 2020 Mar 26.).

High mortality rates, rapid geographic spread of SARS-CoV-2, and not clearly defined etiology of the disease have caused an urgent need to develop effective products for the prevention and treatment of diseases caused by this virus.

Over the last years, multiple efforts have been made for creating various vaccines for coronavirus infections. The developed vaccine candidates can be divided into six classes: 1) viral-vector vaccines; 2) DNA vaccines; 3) subunit vaccines; 4) nano-particles-based vaccines; 5) inactivated whole-virus vaccines; and 6) live attenuated vaccines.

These vaccines were based on selected viral proteins, such as the nucleocapsid (N) protein, envelope (E) protein, NSP16 protein, and coronavirus S protein (Ch. Yong et al. Recent Advances in the Vaccine Development Against Middle East Respiratory Syndrome-Coronavirus. *Front Microbiol*. 2019 Aug 2;10:1781.).

Some of these products have reached the stage of clinical trials (<https://www.clinicaltrials.gov/>). However, these products are not effective against the novel SARS-CoV-2 virus mainly due to a low homology between this coronavirus and SARS-CoV or MERS-CoV. For example, S protein of SARS-CoV-2 and SARS-CoV shows only 76% of homology (Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Sci China Life Sci*. 2020;63(3):457-60). Thus, at the present time not a single registered vaccine is available against the diseases caused by SARS-COV-2.

There is a solution according to patent US7452542B2 which suggests using a live, attenuated Coronaviridae vaccine, wherein the virus is characterized as comprising a genome encoding an ExoN polypeptide comprising a substitution at tyrosine⁶³⁹⁸ of MHV-A59, or an analogous position thereof, and Orf2a polypeptide comprising a substitution at leu¹⁰⁶ of MHV-A59, or an analogous position thereof, and a pharmaceutically acceptable solvent.

There is a solution according to patent WO2016116398A1 which relates to the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) N nucleocapsid protein and/or an

immunogenic fragment thereof, or a nucleic acid molecule encoding the MERS-CoV N nucleocapsid protein and/or the immunogenic fragment thereof, for use as a vaccine.

There is a solution according to patent CN100360557C which describes the use of a S protein of SARS virus, which has mutation in one of the positions: 778D → Y; 77D → G; 244T → I; 1182K → Q; 360F → S; 479N → R или K; 480D → G; 609A → L, to produce vaccine against the severe acute respiratory syndrome. The priority date of filing of patent application: 10.07.2003.

There is a solution according to claim for invention US20080267992A1 which describes the vaccine against severe acute respiratory syndrome based on recombinant human adenovirus serotype 5, containing a sequence of the full-length S protective antigen of the SARS-CoV virus, or a sequence which includes S1 domain of S antigen of the SARS-CoV virus or S2 domain of S antigen of the SARS-CoV virus, or the both domains. In addition, this recombinant virus within the expression cassette contains the human cytomegalovirus promoter (CMV-promoter) and bovine growth hormone polyadenylation (bgh-PolyA) signal.

The authors of the claimed invention chose as a prototype the technical solution according to this patent as the most similar. A significant drawback of this solution is the use of antigens of another species of the family Coronaviridae.

Thus, background of the invention elicits an urgent need for developing a novel immunobiological agent that ensures the induction of effective immune response to the SARS-CoV-2 coronavirus.

Disclosure of the Invention

The aim of the claimed group of inventions is to create an immunobiological agent for the effective induction of immune response to the SARS-CoV-2 virus.

The technical result of the invention is the creation of an effective agent for inducing specific immunity to the SARS-Cov-2.

This technical result is achieved by the creation of an immunobiological agent for the prevention of diseases caused by the severe acute respiratory syndrome virus (SARS-CoV-2) based on recombinant human adenovirus serotype 5, or recombinant human adenovirus serotype 26, containing optimized for the expression in mammalian cells the sequence of S protective antigen of the SARS-CoV-2 virus with gene C'-terminal deletion of 18 amino acids (SEQ ID NO:2).

This technical result is also achieved by the creation of an immunobiological agent for the prevention of diseases caused by the severe acute respiratory syndrome virus (SARS-CoV-2) based on recombinant human adenovirus serotype 5, or recombinant human adenovirus serotype 26, containing optimized for the expression in mammalian cells the SARS-CoV-2 virus full-length S protective antigen sequence and the human IgG1 Fc-fragment sequence (SEQ ID NO:3).

This technical result is also achieved by the creation of an immunobiological agent for the prevention of diseases caused by the severe acute respiratory syndrome (SARS-CoV-2) virus based on recombinant human adenovirus serotype 5, or recombinant human adenovirus serotype 26, containing optimized for the expression in mammalian cells the SARS-CoV-2 virus S protein receptor-binding domain sequence with the viral leader peptide sequence (SEQ ID NO:4).

This technical result is also achieved by the creation of an immunobiological agent for the prevention of diseases caused by the severe acute respiratory syndrome (SARS-CoV-2) virus based on recombinant human adenovirus serotype 5, or recombinant human adenovirus serotype 26, containing optimized for the expression in mammalian cells the SARS-CoV-2 virus protein S receptor-binding domain sequence with the transmembrane domain of vesicular stomatitis virus glycoprotein (SEQ ID NO:5).

This technical result is also achieved by the creation of an immunobiological agent for the prevention of diseases caused by the severe acute respiratory syndrome (SARS-CoV-2) virus based on recombinant human adenovirus serotype 5, or recombinant human adenovirus serotype 26, containing optimized for the expression in mammalian cells the SARS-CoV-2 virus S protein receptor-binding domain sequence with the leader peptide sequence and the human IgG1 Fc-fragment sequence (SEQ ID NO:6).

This technical result is also achieved by the creation of an immunobiological agent for the prevention of diseases caused by the severe acute respiratory syndrome (SARS-CoV-2) virus based on recombinant human adenovirus serotype 5, or recombinant human adenovirus serotype 26, containing optimized for the expression in mammalian cells the SARS-CoV-2 virus full-length S protective antigen sequence on the basis of sequences of S protein genes of the SARS-CoV-2 virus (SEQ ID NO:1) in combination with immunobiological agents (SEQ ID NO:2), and/or (SEQ ID NO:3), and/or (SEQ ID NO:4), and/or (SEQ ID NO:5), and/or (SEQ ID NO:6),.

This technical result is also achieved through the method of induction of specific immunity against the SARS-CoV-2 virus, comprising the administration to mammals of one or more agents (SEQ ID NO:1), and/or (SEQ ID NO:2), and/or (SEQ ID NO:3), and/or (SEQ ID NO:4), and/or (SEQ ID NO:5), and/or (SEQ ID NO:6) in an effective amount.

This technical result is also achieved through the method of induction of specific immunity against the SARS-CoV-2 virus, wherein two different immunobiological agents based on recombinant human adenovirus serotype 5, or two different immunobiological agents based on recombinant human adenovirus serotype 26 are sequentially administered to mammals with a time interval of more than one week.

This technical result is also achieved through the method of induction of specific immunity against the SARS-CoV-2 virus, wherein any of the immunobiological agents based on recombinant human adenovirus serotype 5 and any of the immunobiological agents based on recombinant human adenovirus serotype 26 are sequentially administered to mammals with a time interval of more than one week; or any of the immunobiological agents based on recombinant human adenovirus serotype 26 and any of the immunobiological agents based on recombinant human adenovirus serotype 5 are sequentially administered to mammals with a time interval of more than one week.

This technical result is also achieved through the method of induction of specific immunity against the SARS-CoV-2 virus, wherein any two of the immunobiological agents based on recombinant human adenovirus serotype 5 or serotype 26 are simultaneously administered to mammals.

Essence of the claimed group of inventions may be better understood by reference to drawings, wherein Figures 1 – 5 illustrate the results of assessment of the immunization effectiveness.

The implementation of the invention

Short description of the figures

Fig. 1

illustrates the results of effectiveness assessment of the immunization with the developed immunological agent based on recombinant adenovirus containing optimized for the expression in mammalian cells the protective antigen sequence (of proteins S, RBD, S-del,

S-Fc, RBD-G, RBD-Fc) of SARS-CoV-2 with a sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, as estimated by the percentage of proliferating CD4⁺ lymphocytes re-stimulated by S glycoprotein of the SARS-CoV-2 virus at Day 8 after the immunization of experimental animals.

Y-axis – the number of proliferating cells, %

X-axis – different groups of animals:

- 1) phosphate buffer (100 µl)
- 2) Ad5-S-CoV-2 10⁸ PFU/mouse
- 3) Ad5-S-del-CoV-2 10⁸ PFU/mouse
- 4) Ad5-S-Fc-CoV-2 10⁸ PFU/mouse
- 5) Ad5-RBD-CoV-2 10⁸ PFU/mouse
- 6) Ad5-RBD-G-CoV-2 10⁸ PFU/mouse
- 7) Ad5-RBD-Fc-CoV-2 10⁸ PFU/mouse

Fig. 2

illustrates the results of effectiveness assessment of the immunization with the developed immunological agent based on recombinant adenovirus containing optimized for the expression in mammalian cells the protective antigen sequence (of proteins S, RBD, S-del, S-Fc, RBD-G, RBD-Fc) of SARS-CoV-2 with a sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, as estimated by the percentage of proliferating CD4⁺ lymphocytes re-stimulated by S glycoprotein of the SARS-CoV-2 virus at Day 15 after the immunization of experimental animals.

Y-axis – the number of proliferating cells, %

X-axis – different groups of animals:

- 1) phosphate buffer (100 µl)
- 2) Ad5-S-CoV-2 10⁸ PFU/mouse
- 3) Ad5-S-del-CoV-2 10⁸ PFU/mouse
- 4) Ad5-S-Fc-CoV-2 10⁸ PFU/mouse
- 5) Ad5-RBD-CoV-2 10⁸ PFU/mouse
- 6) Ad5-RBD-G-CoV-2 10⁸ PFU/mouse
- 7) Ad5-RBD-Fc-CoV-2 10⁸ PFU/mouse

Fig. 3

illustrates the results of effectiveness assessment of the immunization with the developed immunological agent based on recombinant adenovirus containing optimized for the expression in mammalian cells the protective antigen sequence (of proteins S, RBD, S-del, S-Fc, RBD-G, RBD-Fc) of SARS-CoV-2 with a sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, as estimated by the percentage of proliferating CD8⁺ lymphocytes re-stimulated by S glycoprotein of the SARS-CoV-2 virus at Day 8 after the immunization of experimental animals.

Y-axis – the number of proliferating cells, %

X-axis – different groups of animals:

- 1) phosphate buffer (100 µl)
- 2) Ad5-S-CoV-2 10⁸ PFU/mouse
- 3) Ad5-S-del-CoV-2 10⁸ PFU/mouse
- 4) Ad5-S-Fc-CoV-2 10⁸ PFU/mouse
- 5) Ad5-RBD-CoV-2 10⁸ PFU/mouse
- 6) Ad5-RBD-G-CoV-2 10⁸ PFU/mouse
- 7) Ad5-RBD-Fc-CoV-2 10⁸ PFU/mouse

Fig. 4

illustrates the results of effectiveness assessment of the immunization with the developed immunological agent based on recombinant adenovirus containing optimized for the expression in mammalian cells the protective antigen sequence (of proteins S, RBD, S-del, S-Fc, RBD-G, RBD-Fc) SARS-CoV-2 with a sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, as estimated by the percentage of proliferating CD8⁺ lymphocytes re-stimulated by S glycoprotein of the SARS-CoV-2 virus at Day15 after the immunization of experimental animals.

Y-axis – the number of proliferating cells, %

X-axis – different groups of animals:

- 1) phosphate buffer (100 µl)
- 2) Ad5-S-CoV-2 10⁸ PFU/mouse
- 3) Ad5-S-del-CoV-2 10⁸ PFU/mouse
- 4) Ad5-S-Fc-CoV-2 10⁸ PFU/mouse

- 5) Ad5-RBD-CoV-2 10^8 PFU/mouse
- 6) Ad5-RBD-G-CoV-2 10^8 PFU/mouse
- 7) Ad5-RBD-Fc-CoV-2 10^8 PFU/mouse

Fig. 5

illustrates the results of effectiveness assessment of the developed immunobiological agent based on recombinant adenovirus containing optimized for the expression in mammalian cells the protective antigen sequence (of proteins S, RBD, S-del, S-Fc, RBD-G, RBD-Fc) of SARS-CoV-2 with a sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, as estimated by increase in IFN-gamma concentration in the medium after the splenocytes of C57/BL6 mice, immunized with the adenoviral constructs, were stimulated with the SARS-CoV-2 virus full-length S protein, at Day15 after the immunization of experimental animals.

Y-axis – the values of increase in IFN-gamma concentration in the medium with stimulated cells compared with intact cells (-fold).

X-axis – studied groups of animals: intact animals and animals with administered 10^8 PFU/mouse

- 1) phosphate buffer (100 μ l)
- 2) Ad5-S-CoV-2 10^8 PFU/mouse
- 3) Ad5-S-del-CoV-2 10^8 PFU/mouse
- 4) Ad5-S-Fc-CoV-2 10^8 PFU/mouse
- 5) Ad5-RBD-CoV-2 10^8 PFU/mouse
- 6) Ad5-RBD-G-CoV-2 10^8 PFU/mouse
- 7) Ad5-RBD-Fc-CoV-2 10^8 PFU/mouse

The first stage in the development of immunobiological agent against the SARS-CoV-2 coronavirus was the selection of a vaccine antigen. As a part of this process, the literature search was performed which demonstrated that the coronavirus S protein was the most promising antigen for creating a candidate vaccine. Type 1 transmembrane glycoprotein is responsible for virus particles binding, fusion and entry into the cells. As demonstrated, it was an inducer of neutralizing antibodies (Liang M et al, SARS patients-derived human recombinant antibodies to S and M proteins efficiently neutralize SARS-coronavirus infectivity. Biomed Environ Sci. 2005 Dec;18(6):363-74).

The S protein consists of a signal peptide (amino acids 1-12) and 3 domains: an extracellular domain (amino acids 13-1193), transmembrane domain (amino acids 1194-1215), and an intracellular domain (amino acids 1216-1255). The extracellular domain consists of two subunits S1 and S2, and a small region between them, whose functions are not fully understood. The S1 subunit is responsible for binding the virus to ACE2 (angiotensin-converting enzyme 2) receptor. A fragment located in the middle region of the S1 subunit (amino acids 318-510) has been named the receptor-binding domain (RBD). The S2 subunit which contains a putative fusion peptide and two heptad repeats (HR1 and HR2) promotes the fusion of the virus and the target cell membrane. The infection is initiated by the viral S1 subunit binding through its RBD to the ACE2 cell receptor.

Next, a fusion core between HR1 and HR2 regions of the S2 subunit is formed. As a result, the viral and cellular membranes get into close proximity followed by their fusion and the virus enters the cell. Therefore, the use of S protein or its fragment in a vaccine formula may induce antibodies that inhibit the virus entry into the cell.

To achieve the most effective induction of immune response, the authors claimed multiple variants of modification of this antigen, as well as its potential combination with the transmembrane domain of vesicular stomatitis virus glycoprotein for increasing the level of expression of the target protein.

Six different variants of nucleotide sequences were obtained (of modified S gene of the SARS-CoV-2 virus, or the receptor-binding domain of S protein) by optimizing these sequences for expression in mammalian cells.

Then, multiple constructs based on recombinant human adenovirus serotype 5 or serotype 26 were developed to ensure that the modified genes are delivered effectively to mammalian cells. The adenoviral vectors were selected, since they have such advantages as the safety, broad tissue tropism, well-characterized genome, simplicity of genetic manipulations, capability to integrate large transgenic DNA inserts, intrinsic adjuvant properties, and the ability to induce stable T-cell-mediated and humoral immune response.

Human adenoviruses of serotype 5 are the best studied ones among the known adenoviruses, and therefore they are most commonly used in gene therapy for deriving vectors. Technologies were developed to produce first- and second-generation vectors, chimeric viral vectors (containing proteins of other viral serotypes) (J.N. Glasgow et. al., An adenovirus vector with a chimeric fiber derived from canine adenovirus type 2 displays novel tropism, *Virology*, 2004, № 324, 103–116), and multiple other vectors. Also, vectors derived from other serotypes were produced (H. Chen et. al., *Adenovirus-Based Vaccines*:

Comparison of Vectors from Three Species of Adenoviridae, *Virology*, 2010, № 84(20), 10522–10532).

Vectors based on human adenovirus serotype 26 demonstrate a high level of immunogenicity in primates, where they are able to induce a strong CD8⁺ T-cell response which, in terms of quality, is superior to T-cell-mediated response elicited in the host body by vectors based on human adenovirus serotype 5 (J. Liu et. al., Magnitude and phenotype of cellular immune responses elicited by recombinant adenovirus vectors and heterologous prime-boost regimens in rhesus monkeys, *Virology*, 2008, № 82, 4844–4852). With that, more epitopes are recognized and the production of a broader spectrum of factors (rather than a predominant production of gamma-interferon) is induced (J. Liu et. al., Magnitude and phenotype of cellular immune responses elicited by recombinant adenovirus vectors and heterologous prime-boost regimens in rhesus monkeys, *Virology*, 2008, № 82, 4844–4852). These data suggest that human adenovirus serotype-26-based vectors have fundamental differences as regards their ability to induce immune response to the target antigen as compared with other adenoviral vectors.

Variant 1 invention is the recombinant human adenovirus serotype 5, or the recombinant human adenovirus serotype 26, containing optimized for the expression in mammalian cells the sequence of full-length protective S antigen of the SARS-CoV-2 virus based on S protein gene sequences of the SARS-CoV-2 virus (SEQ ID NO:1).

Variant 2 invention is the recombinant human adenovirus serotype 5, or the recombinant human adenovirus serotype 26 containing optimized for the expression in mammalian cells the sequence of full-length S protective antigen of the SARS-CoV-2 virus with gene C'-terminal deletion of 18 amino acids (SEQ ID NO:2).

Variant 3 invention is the recombinant human adenovirus serotype 5, or the recombinant human adenovirus serotype 26 containing optimized for the expression in mammalian cells the sequence of full-length S protective antigen of the SARS-CoV-2 virus and the human IgG1 Fc-fragment sequence (SEQ ID NO:3).

Variant 4 invention is the recombinant human adenovirus serotype 5, or the recombinant human adenovirus serotype 26 containing optimized for the expression in mammalian cells the SARS-CoV-2 virus S protein receptor-binding domain sequence with the viral leader peptide sequence (SEQ ID NO:4).

Variant 5 invention is the recombinant human adenovirus serotype 5, or the recombinant human adenovirus serotype 26, containing optimized for the expression in

mammalian cells the SARS-CoV-2 virus S protein receptor-binding domain sequence with the transmembrane domain of vesicular stomatitis virus glycoprotein (SEQ ID NO:5).

Variant 6 invention is the recombinant human adenovirus serotype 5, or the recombinant human adenovirus serotype 26 containing optimized for the expression in mammalian cells the SARS-CoV-2 virus S protein receptor-binding domain sequence with the leader peptide sequence and the human IgG1 Fc-fragment sequence (SEQ ID NO:6).

The authors have developed a method for inducing specific immunity to the SARS-CoV-2 virus, which involves the administration to mammals of one or more agents from variants 1-6 in an effective amount. This method envisages:

- 1) sequential administration to mammals of two different immunobiological agents based on recombinant human adenovirus serotype 5 or two different immunobiological agents based on recombinant human adenovirus serotype 26 presented herein in variants 1-6 with a time interval of more than one week.
- 2) sequential administration to mammals of any of the immunobiological agents based on recombinant human adenovirus serotype 5 and any of the immunobiological agents based on recombinant human adenovirus serotype 26 presented herein in variants 1-6 with a time interval of more than one week, or sequential administration to mammals of any of the immunobiological agents based on recombinant human adenovirus serotype 26 and any of the immunobiological agents based on recombinant human adenovirus serotype 5 presented herein in variants 1-6 with a time interval of more than one week.
- 3) simultaneous administration to mammals of any two immunobiological agents based on recombinant human adenovirus serotype 5 or serotype 26 presented herein in claim 1 and/or claim 2, and/or claim 3, and/or claim 4, and/or claim 5, and/or claim 6.

The implementation of the invention is proven by the following examples:

Example 1. Obtaining of different variants of the SARS-CoV-2 virus S glycoprotein

At the first stage, the authors developed several modifications of the vaccine antigen to achieve the most effective immune response.

As a basis the SARS-CoV-2 virus S protein with a sequence SEQ ID NO:1 was taken which was then modified by several methods:

1) In order to present S protein on the plasma membrane, the deletion of 18 amino acids on gene C'-terminal (S-del) SEQ ID NO:2 was performed (used for variant 2).

2) Also, the SARS-CoV-2 virus full-length S protective antigen sequence, optimized for the expression in mammalian cells, with the human IgG1 Fc-fragment sequence was obtained (used for variant 3).

This modification enhances immunogenicity through a potential binding of protein Fc fragment to Fc receptor in antigen presentation cells (Li Z., Palaniyandi S., Zeng R., Tuo W., Roopenian D.C., Zhu X., Transfer of IgG in the female genital tract by MHC class I-related neonatal Fc receptor (FcRn) confers protective immunity to vaginal infection. Proc. Natl. Acad. Sci. U.S.A., 2011, №108, 4388–93), and also increases the protein stability and prolongs its half-life *in vivo* (Zhang M.Y., Wang Y., Mankowski M.K., Ptak R.G., Dimitrov D.S., Cross-reactive HIV-1-neutralizing activity of serum IgG from a rabbit immunized with gp41 fused to IgG1 Fc: Possible role of the prolonged half-life of the immunogen, Vaccine, 2009, №27, 857–863).

3) To assess the immunogenicity solely of the receptor-binding domain (RBD) of the SARS-CoV-2 virus S protein in a secreted form, a sequence SEQ ID NO:4 (used for variant 4) was created which contains the S protein receptor-binding domain sequence with the leader peptide sequence (added for protein secretion).

4) To investigate the SARS-CoV-2 virus S protein RBD in a non-secreted form, a sequence SEQ ID NO:5 was selected (used for variant 5) consisting of the SARS-CoV-2 virus S protein RBD to which the sequence of transmembrane domain of vesicular stomatitis virus glycoprotein (RBD-G) was added.

5) To investigate a secreted form of the S protein RBD with the leader peptide sequence and the human IgG1 Fc-fragment sequence, a sequence SEQ ID NO:6 was selected (used for variant 6). The addition of the human IgG1 Fc-fragment sequence enhances immunogenicity through a potential binding of protein Fc fragment to Fc receptor in antigen presentation cells (Z. Li et. al., Transfer of IgG in the female genital tract by MHC class I-related neonatal Fc receptor (FcRn) confers protective immunity to vaginal infection, Proceedings of the National Academy of Sciences USA, 2011, № 108, 4388–4393), and also may increase the protein stability and prolong its half-life *in vivo* (M.Y. Zhang et. al., Crossreactive HIV-1-neutralizing activity of serum IgG from a rabbit immunized with gp41

fused to IgG1 Fc: Possible role of the prolonged half-life of the immunogen, Vaccine, 2008, № 27, 857–63).

Example 2. Obtaining of genetic constructs encoding the S protein gene in different variants.

At the next stage, amino acid sequences presented herein in example 1 (SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6) were translated to nucleotide sequences. Next step comprised the optimization of obtained sequences for the expression in mammalian cells. All nucleotide sequences were obtained using the method of synthesis of the ZAO “Evrogen” Company (Moscow). As a result, the following genetic constructs were available:

- 1) pVax-S-CoV-2, containing nucleotide sequence of the SARS-CoV-2 virus full-length S gene;
- 2) pVax-S-del-CoV-2, containing nucleotide sequence of the SARS-CoV-2 virus S gene with deletion of 18 amino acids at the gene C'-terminal;
- 3) pVax-S-Fc-CoV-2, containing nucleotide sequence of the SARS-CoV-2 virus full-length S gene and sequence of the human IgG1 Fc-fragment;
- 4) pAL2-T-RBD-CoV-2, containing nucleotide sequence of the S protein receptor-binding domain with the leader peptide gene sequence;
- 5) pAL2-T-RBD-G-CoV-2, containing nucleotide sequence of the S protein receptor-binding domain with G gene of the vesicular stomatitis virus;
- 6) pAL2-T-RBD-Fc-CoV-2, containing nucleotide sequence of the S protein receptor-binding domain with the leader peptide gene sequence, and nucleotide sequence of the human IgG1 Fc-fragment.

Then, by genetic engineering methods, the S protein gene sequence from construct pVax-S-CoV-2 was cloned, using XbaI restriction endonuclease, into a shuttle plasmid pShuttle-CMV (Stratagene, US); and, the obtained plasmid was named pShuttle-S-CoV-2. Thus, the shuttle plasmid pShuttle-S-CoV-2 was created which carries the nucleotide sequence of S amino acid sequence (SEQ ID NO:1), optimized for the expression in mammalian cells (as obtained in example 1).

Similarly, the nucleotide sequences of modified variants of the SARS-CoV-2 virus S protein were cloned into a shuttle plasmid pShuttle-CMV (Stratagene, US) and the following shuttle plasmids were obtained:

- pShuttle-S-del-CoV-2 (contains the optimized nucleotide sequence of the SARS-CoV-2 virus S gene with deletion of 18 amino acids at the gene C'-terminal);
- pShuttle-S-Fc-CoV-2, containing the optimized nucleotide sequence of the SARS-CoV-2 virus full-length S gene and the sequence of Fc-fragment from human IgG1;
- pShuttle-RBD-CoV-2 (contains the optimized nucleotide sequence of the SARS-CoV-2 virus S receptor-binding domain);
- pShuttle-RBD-G-CoV-2 (contains the optimized nucleotide sequence of the SARS-CoV-2 virus S receptor-binding domain with the transmembrane domain of the vesicular stomatitis virus glycoprotein);
- pShuttle-RBD-Fc-CoV-2 (contains the optimized nucleotide sequence of the SARS-CoV-2 virus S receptor-binding domain with the optimized sequence of Fc-fragment from human IgG1).

Example 3. Obtaining of an immunobiological agent based on recombinant human adenovirus serotype 5.

At the next stage, a recombinant adenoviral plasmid pAd5-S-CoV-2 was obtained which contains the sequence of SARS-CoV-2 full-length S protective antigen (SEQ ID NO:1) (variant 1) optimized for the expression in mammalian cells. This plasmid was obtained by the process of homologous recombination between the plasmid pAd containing the genomic region of human adenovirus serotype 5 with deleted E1 and E3 sites, and the shuttle plasmid pShuttle-S (obtained in example 3) which carries homologous sites of the adenovirus genome and an expression cassette with the target gene (of S protein). For this purpose, the shuttle plasmid pShuttle-S obtained in example 3 was linearized by restriction endonuclease PmeI.

Homologous recombination was performed in the cells of *E. coli* strain BJ5183. Plasmid pAd was mixed with plasmid pShuttle-S, and then the received mixture was used to transform the *E. coli* cells by electroporation method according to the Guide "MicroPulser™ Electroporation Apparatus Operating Instructions and Applications Guide" (Bio-Rad, US). As the transformation was completed, the cells of *E. coli* strain BJ5183 were inoculated in LB-agar dishes, containing a selective antibiotic, and grown for 18 hours at +37°C. A transformation effectiveness was $10^{10} - 10^{11}$ transformed clones per μg of plasmid pBluescript II SK(-).

As a result of homologous recombination, a cassette with the target transgene (of S protein) appeared in the plasmid pAd, and the antibiotic-resistance gene changed.

Thus, the recombinant adenoviral plasmid pAd5-S-CoV-2 was constructed which contains a full-length genome of recombinant human adenovirus serotype 5 (with E1 and E3 sites deleted from the genome) with the integrated genetic construct obtained in example 3. Then, the plasmid pAd5-S-CoV-2 was hydrolyzed with the restriction endonuclease Pac I and used for the transfection of permissive cell culture of human embryonic kidney cell line HEK 293. The genome of HEK 293 cells contains the integrated E1 site of human adenovirus serotype 5 genome, so that the replication of recombinant replication-defective human adenoviruses serotype 5 may occur. At day 6 after the transfection, the first blind passages were performed to ensure a more effective production of the recombinant adenovirus. Upon occurrence of the cytopathic virus effect (microscopy data), the cells with culture medium were frozen for three times to facilitate the disruption of cells and the virus release. The obtained material was then used for the accumulation of preparative amounts of the recombinant adenoviruses.

Activity of the preparation pAd5-S-CoV-2 hereinafter was assessed by the standard titration technique in the culture of susceptible 293 HEK cells using a plaque forming cell assay.

In order to verify the creation of a construct of potential recombinant pseudo-adenoviral particle derived from human adenovirus serotype 5, expressing the SARS-CoV-2 virus S gene, the polymerase chain reaction (PCR) was performed according to the established standard technique.

In a similar way, additional five recombinant viruses were obtained: Ad5-S-del-CoV-2, Ad5-S-Fc-CoV-2, Ad5-RBD-CoV-2, Ad5-RBD-G-CoV-2, Ad5-RBD-Fc-CoV-2.

Thus, as a result, variants of the immunobiological agent based on recombinant human adenovirus serotype 5 were obtained, containing:

- 1) optimized nucleotide sequence of the SARS-CoV-2 virus S receptor-binding domain (variant 1);
- 2) optimized nucleotide sequence of the SARS-CoV-2 virus S protective antigen with deletion of 18 amino acids at the gene C'-terminal (variant 2);
- 3) sequence of the SARS-CoV-2 virus full-length S protective antigen and sequence of the human IgG1 Fc-fragment optimized for the expression in mammalian cells (variant 3);

- 4) optimized nucleotide sequence of the S protein receptor-binding domain with the leader peptide sequence (variant 4);
- 5) optimized nucleotide sequence of the S protein receptor-binding domain with the transmembrane domain of vesicular stomatitis virus glycoprotein (variant 5),
- 6) optimized sequence of the S protein receptor-binding domain with the leader peptide sequence and the human IgG1 Fc-fragment sequence (variant 6).

Example 4. Obtaining of an immunobiological agent based on recombinant human adenovirus serotype 26.

At the first stage, an expression cassette with the SARS-CoV-2 virus S gene was placed in the recombinant vector pAd26-ORF6-Ad5. For this purpose, the vector pAd26-ORF6-Ad5 was linearized with the restriction endonuclease PmeI, while the plasmid construct pShuttle-S, obtained in example 3, was processed with the restriction endonucleases PmeI. Hydrolysis products were ligated, and then the plasmid pAd26-S-CoV-2 was produced using standard techniques.

At the next stage, the plasmid pAd26-S-CoV-2 was hydrolyzed with the restriction endonucleases PacI and SwaI and used for the transfection of permissive cell line HEK 293 culture. On the third day after the transfection, the first blind passages were performed to ensure a more effective generation of recombinant virus. Upon occurrence of the cytopathic virus effect (microscopy data), the cells with culture medium were frozen for three times to facilitate the disruption of cells and the virus release. The obtained material was then used for the accumulating preparative amounts of recombinant adenoviruses. Activity of the preparation pAd26-S-CoV-2 hereinafter was assessed by the standard titration technique in the culture of 293 HEK cells using a plaque forming cell assay.

In order to verify that construct of the proposed recombinant pseudo-adenoviral particle based on human adenovirus serotype 26, expressing the SARS-CoV-2 virus S gene, has been generated, polymerase chain reaction (PCR) was performed according to the established standard technique.

In a similar way, additional five recombinant viruses were obtained: pAd26-S-dek-CoV-2, pAd26-S-Fc-CoV-2, pAd26-RBD-CoV-2, pAd26-RBD-G-CoV-2, pAd26-RBD-Fc-CoV-2.

Thus, as a result, variants of the immunobiological agent based on recombinant human adenovirus serotype 26 were obtained, containing:

- 1) optimized nucleotide sequence of the SARS-CoV-2 virus S receptor-binding domain (variant 1);
- 2) optimized nucleotide sequence of the SARS-CoV-2 virus S protective antigen with deletion of 18 amino acids at the gene C'-terminal (variant 2);
- 3) sequence of the SARS-CoV-2 virus full-length S protective antigen and sequence of the human IgG1 Fc-fragment optimized for the expression in mammalian cells (variant 3);
- 4) optimized nucleotide sequence of the S protein receptor-binding domain with the leader peptide sequence (variant 4);
- 5) optimized nucleotide sequence of the S protein receptor-binding domain with the transmembrane domain of vesicular stomatitis virus glycoprotein (variant 5);
- 6) optimized sequence of the S protein receptor-binding domain with the leader peptide sequence and the human IgG1 Fc-fragment sequence (variant 6).

Example 5. Verification of the expression of different variants of S glycoprotein gene of the SARS-CoV-2 virus in HEK293 cells after the addition of immunobiological agent based on recombinant human adenovirus serotype 5.

The aim of this experiment was to verify the ability of constructed recombinant adenoviruses Ad5-S-CoV-2, Ad5-S-del-CoV-2, Ad5-S-Fc-CoV-2, Ad5-RBD-CoV-2, Ad5-RBD-G-CoV-2, Ad5-RBD-Fc-CoV-2 to express different variants of S protein gene in mammalian cells.

HEK293 cells were cultured in DMEM medium containing 10% fetal calf serum in incubator at 37°C and 5% CO₂. The cells were placed in 35mm² culture Petri dishes and incubated for 24 hours until reaching 70% confluence. Then, the studied preparations of recombinant adenoviruses (Ad5-S-CoV-2, Ad5-S-del-CoV-2, Ad5-S-Fc-CoV-2, Ad5-RBD-CoV-2, Ad5-RBD-G-CoV-2, Ad5-RBD-Fc-CoV-2), and control preparation (Ad5-null – recombinant adenovirus containing no inserts) in an amount of 100 PFU/cell and phosphate buffer saline (PBS), as a negative control, were added to the cells. Two days after the transduction, the cells were collected and lysed in 0.5 ml of normal strength buffer CCLR (Promega). The lysate was diluted with carbonate-bicarbonate buffer and placed in ELISA plate wells. The plate was incubated over the night at +4°C.

The plate wells were washed for three times with normal strength washing buffer at an amount of 200 μ l per well, and then 100 μ l of blocking buffer were added to every well; the plate was covered with a lid and incubated for 1 hour at 37°C in shaker at 400 rpm. Then, the plate wells were washed for three times with normal strength buffer at an amount of 200 μ l per well and 100 μ l of convalescent blood serum were added to every well. The plate was covered with a lid and incubated at room temperature in shaker at 400 rpm for 2 hours. Then, the plate wells were washed for three times with normal strength washing buffer at an amount of 200 μ l per well, and then 100 μ l of secondary antibodies conjugated with biotin were added. The plate was covered with a lid and incubated at room temperature in shaker at 400 rpm for 2 hours. Next, a solution of streptavidin conjugated with horseradish peroxidase was prepared. For this purpose the conjugate in the amount of 60 μ l was diluted in 5.94 ml of an assay buffer. The plate wells were washed twice with normal strength washing buffer at an amount of 200 μ l per well and 100 μ l of streptavidin solution conjugated with horseradish peroxidase were added to each of the plate wells. The plate was incubated at room temperature in shaker at 400 rpm for 1 hour. Then, the plate wells were washed twice with normal strength washing buffer at an amount of 200 μ l per well and 100 μ l of TMB substrate were added to each of the plate wells. The plate was incubated under darkness at room temperature for 10 minutes, then 100 μ l of stop solution were added to each of the plate wells. The optical density was measured using plate spectrophotometer (Multiskan FC, Thermo) at a wavelength of 450 nm. The experiment results are presented in Table 1.

Table 1 – Results of the experiment for verifying the expression of different variants of S glycoprotein gene of the SARS-CoV-2 virus in HEK293 cells after the addition of immunobiological agent based on recombinant human adenovirus serotype 5.

	Mean optical density at a wavelength of 450 nm
PBS	0.19 ($\pm$ 0.05)
Ad5-null	0.23 ($\pm$ 0.08)
Ad5-S-CoV-2	1.85 ($\pm$ 0.15)
Ad5-S-del-CoV-2	1.63 ($\pm$ 0.19)
Ad5-S-Fc-CoV-2	1.57 ($\pm$ 0.30)
Ad5-RBD-CoV-2	1.47 ($\pm$ 0.21)
Ad5-RBD-G-CoV-2	1.52 ($\pm$ 0.19)
Ad5-RBD-Fc-CoV-2	1.58 $\pm$ (0.11)

As shown by the received data, the expression of different variants of the target protein was observed in all cells transduced with recombinant adenoviruses Ad5-S-CoV-2, Ad5-S-del-CoV-2, Ad5-S-Fc-CoV-2, Ad5-RBD-CoV-2, Ad5-RBD-G-CoV-2, Ad5-RBD-Fc-CoV-2.

Example 6. Verification of the expression of different variants of S glycoprotein gene of the SARS-CoV-2 virus in HEK293 cells after the addition of immunobiological agent based on recombinant human adenovirus serotype 26.

The aim of this experiment was to verify the ability of constructed recombinant adenoviruses pAd26-S-CoV-2, Ad26-S-del-CoV-2, Ad26-S-Fc-CoV-2, pAd26-RBD-CoV-2, pAd26-RBD-G-CoV-2, pAd26-RBD-Fc-CoV-2 to express different variants of S protein gene in mammalian cells.

HEK293 cells were cultured in DMEM medium containing 10% fetal calf serum in incubator at 37°C and 5% CO₂. The cells were placed in 35mm² culture Petri dishes and incubated for 24 hours until reaching 70% confluence. Then, the studied preparations of recombinant adenoviruses (pAd26-S-CoV-2, Ad26-S-del-CoV-2, Ad26-S-Fc-CoV-2, pAd26-RBD-CoV-2, pAd26-RBD-G-CoV-2, pAd26-RBD-Fc-CoV-2), and control preparation (Ad26-null – recombinant adenovirus containing no inserts) in an amount of 100 PFU/cell and phosphate buffer saline (PBS), as a negative control, were added to the cells. Two days after the transduction, the cells were collected and lysed in 0.5 ml of normal strength buffer CCLR (Promega). The lysate was diluted with carbonate-bicarbonate buffer and placed in ELISA plate wells. The plate was incubated over the night at +4°C.

The plate wells were washed for three times with normal strength washing buffer at an amount of 200 µl per well, and then 100 µl of blocking buffer were added to each well; the plate was covered with a lid and incubated for 1 hour at 37°C in shaker at 400 rpm. Then, the plate wells were washed for three times with normal strength buffer at an amount of 200 µl per well and 100 µl of convalescent blood serum was added to every well. The plate was covered with a lid and incubated at room temperature in shaker at 400 rpm for 2 hours. Then, the plate wells were washed for three times with normal strength washing buffer at an amount of 200 µl per well, and 100 µl of secondary antibodies conjugated with biotin were added. The plate was covered with a lid and incubated at room temperature in shaker at 400 rpm for 2 hours. Next, solution of streptavidin conjugated with horseradish peroxidase was prepared.

For this purpose, the conjugate in the amount of 60 μ l was diluted in 5.94 ml of assay buffer. The plate wells were washed twice with normal strength washing buffer at an amount of 200 μ l per well and 100 μ l of streptavidin solution conjugated with horseradish peroxidase were added to each of the plate wells. The plate was incubated at room temperature in shaker at 400 rpm for 1 hour. Then, the plate wells were washed twice with normal strength washing buffer at an amount of 200 μ l per well and 100 μ l of TMB substrate were added to each of the plate wells and incubated under darkness at room temperature for 10 minutes. Then 100 μ l of stop solution was added to each of the plate wells. The value of optical density was measured using plate spectrophotometer (Multiskan FC, Thermo) at a wavelength of 450 nm. The experiment results are presented in Table 2.

Table 2 – Results of the experiment for verifying the expression of different variants of S glycoprotein gene of the SARS-CoV-2 virus in HEK293 cells after the addition of immunobiological agent based on recombinant human adenovirus serotype 26.

	Mean optical density at a wavelength of 450 nm
PBS	0.17 ($\pm$ 0.08)
Ad26-null	0.22 ($\pm$ 0.09)
Ad26-S-CoV-2	1.68 ($\pm$ 0.21)
Ad26-S-del-CoV-2	1.65 (0.14)
Ad26-S-Fc-CoV-2	1.71 ($\pm$ 0.13)
Ad26-RBD-CoV-2	1.61 ($\pm$ 0.18)
Ad26-RBD-G-CoV-2	1.45 ($\pm$ 0.22)
Ad26-RBD-Fc-CoV-2	1.51 $\pm$ (0.14)

As shown by the received data, the expression of different variants of the target protein was observed in all cells transduced with recombinant adenoviruses pAd26-S-CoV-2, Ad26-S-del-CoV-2, Ad26-S-Fc-CoV-2, pAd26-RBD-CoV-2, pAd26-RBD-G-CoV-2, pAd26-RBD-Fc-CoV-2.

Example 7. A method of utilization of the developed immunobiological agent by a single administration to mammals in an effective amount for the induction of specific immunity to SARS-CoV-2.

The developed immunobiological agent based on recombinant human adenoviruses serotypes 5 and 26, containing optimized for the expression in mammalian cells the protective antigen sequence (of proteins S, S-del, S-Fc, RBD, RBD-G, RBD-Fc) of SARS-CoV-2 with a sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 is utilized by administering to mammals through any of the administration routes known for this viral vector (subcutaneously, intramuscularly, intravenously, intranasally). This way, immune response to the target protein of SARS-CoV-2 glycoprotein develops in mammals.

One of the main characteristics of immunization effectiveness is an antibody titer. Example presents data relating to changes in the titer of antibodies against SARS-CoV-2 glycoprotein at day 21 after a single intramuscular immunization of animals with the immunobiological agent, containing the recombinant human adenovirus of serotype 5 or 26, comprising optimized for the expression in mammalian cells the sequence of protective antigen (of proteins S, S-del, S-Fc, RBD, RBD-G, RBD-Fc) of SARS-CoV-2 with a sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6.

Mammals used in the experiment — mice C57BL/6, females, 18 g. All the animals were divided into 43 groups, 5 animals each, injected intramuscularly with:

- 1) Ad5-S-CoV-2 10^7 PFU/mouse
- 2) Ad5-S-del-CoV-2 10^7 PFU/mouse
- 3) Ad5-S-Fc-CoV-2 10^7 PFU/mouse
- 4) Ad5-RBD-CoV-2 10^7 PFU/mouse
- 5) Ad5-RBD-G-CoV-2 10^7 PFU/mouse
- 6) Ad5-RBD-Fc-CoV-2 10^7 PFU/mouse
- 7) Ad5-null 10^7 PFU/mouse
- 8) Ad5-S-CoV-2 10^8 PFU/mouse
- 9) Ad5-S-del-CoV-2 10^8 PFU/mouse
- 10) Ad5-S-Fc-CoV-2 10^8 PFU/mouse
- 11) Ad5-RBD-CoV-2 10^8 PFU/mouse
- 12) Ad5-RBD-G-CoV-2 10^8 PFU/mouse
- 13) Ad5-RBD-Fc-CoV-2 10^8 PFU/mouse
- 14) Ad5-null 10^8 PFU/mouse
- 15) Ad5-S-CoV-2 10^9 PFU/mouse
- 16) Ad5-S-del-CoV-2 10^9 PFU/mouse

- 17) Ad5-S-Fc-CoV-2 10^9 PFU/mouse
- 18) Ad5-RBD-CoV-2 10^9 PFU/mouse
- 19) Ad5-RBD-G-CoV-2 10^9 PFU/mouse
- 20) Ad5-RBD-Fc-CoV-2 10^9 PFU/mouse
- 21) Ad5-null 10^9 PFU/mouse
- 22) Ad26-S-CoV-2 10^7 PFU/mouse
- 23) Ad26-S-del-CoV-2 10^7 PFU/mouse
- 24) Ad26-S-Fc-CoV-2 10^7 PFU/mouse
- 25) Ad26-RBD-CoV-2 10^7 PFU/mouse
- 26) Ad26-RBD-G-CoV-2 10^7 PFU/mouse
- 27) Ad26-RBD-Fc-CoV-2 10^7 PFU/mouse
- 28) Ad26-null 10^7 PFU/mouse
- 29) Ad26-S-CoV-2 10^8 PFU/mouse
- 30) Ad26-S-del-CoV-2 10^8 PFU/mouse
- 31) Ad26-S-Fc-CoV-2 10^8 PFU/mouse
- 32) Ad26-RBD-CoV-2 10^8 PFU/mouse
- 33) Ad26-RBD-G-CoV-2 10^8 PFU/mouse
- 34) Ad26-RBD-Fc-CoV-2 10^8 PFU/mouse
- 35) Ad26-null 10^8 PFU/mouse
- 36) Ad26-S-CoV-2 10^9 PFU/mouse
- 37) Ad26-S-del-CoV-2 10^9 PFU/mouse
- 38) Ad26-S-Fc-CoV-2 10^9 PFU/mouse
- 39) Ad26-RBD-CoV-2 10^9 PFU/mouse
- 40) Ad26-RBD-G-CoV-2 10^9 PFU/mouse
- 41) Ad26-RBD-Fc-CoV-2 10^9 PFU/mouse
- 42) Ad26-null 10^9 PFU/mouse
- 43) phosphate buffer saline

Three weeks later, blood samples were taken from the tail vein of animals, and blood serum was isolated. Antibody titers were determined by ELISA using the following protocol:

- 1) Protein (S) was adsorbed onto wells of 96-well ELISA plate for 16 hours at +4°C.
- 2) Then, for preventing a non-specific binding, the plate was “blocked” with 5% milk dissolved in TPBS in an amount of 100 μ l per well. It was incubated in shaker at 37°C for one hour.

3) Serum samples from the immunized mice were diluted using a 2-fold dilution method. Totally, 12 dilutions of each sample were prepared.

4) 50 μ l of each of the diluted serum samples were added to the plate wells.

5) Then, incubation at 37°C for 1 hour was performed.

6) After incubation the wells were washed three times with phosphate buffer.

7) Then, secondary antibodies against mouse immunoglobulins conjugated with horseradish peroxidase were added.

8) Then, incubation at 37°C for 1 hour was performed.

9) After incubation the wells were washed three times with phosphate buffer.

10) Then, tetramethylbenzidine (TMB) solution was added which serves as a substrate for horseradish peroxidase and is converted into a colored compound by the reaction. The reaction was stopped after 15 minutes by the adding sulfuric acid. Next, using a spectrophotometer the optical density (OD) of the solution was measured in each well at a wavelength of 450 nm.

Antibody titer was determined as the last dilution at which the optical density of the solution was significantly higher than in the negative control group. The obtained results (geometric mean) are presented in Table 3.

Table 3 – Antibody titers against S protein in the blood serum of mice (geometric mean of antibody titers)

Table 3.

Recombinant adenovirus	PFU/mouse		
	10^7	10^8	10^9
Ad5-null	0	0	0
Ad5-S-CoV-2	1:10809	1:18820	1:57052
Ad5-S-del-CoV-2	1:21619	1:28526	1:114105
Ad5-S-Fc-CoV-2	1:14263	1:18820	1:57052
Ad5-RBD-CoV-2	1:12417	1:14263	1:99334
Ad5-RBD-G-CoV-2	1:32768	1: 49667	1:172951
Ad5-RBD-Fc-CoV-2	1:10809	1:12417	1:28526
Ad26-null	0	0	0
Ad26-S-CoV-2	1:18820	1:24834	1:43238
Ad26-S-del-CoV-2	1:24834	1:43238	1:57052

Ad26-S-Fc-CoV-2	1:28526	1:32768	1:86475
Ad26-RBD-CoV-2	1:12417	1:18820	1:86475
Ad26-RBD-G-CoV-2	1:24834	1:32768	1:150562
Ad26-RBD-Fc-CoV-2	1:9410	1:12417	1:24834

The results of experiment have shown that the developed immunobiological agent administered to mammals induces humoral immune response to SARS-CoV-2 glycoprotein over the entire selected dose range. It is obvious that dose escalation will result in antibody titer increase in the mammalian blood till the toxic effect occurs.

Example 8. A method of utilization of the developed immunobiological agent by sequential administration to mammals in an effective amount for the induction of specific immunity to SARS-CoV-2.

This example describes a method of utilization of the developed immunobiological agent based on recombinant human adenoviruses serotype 5, containing optimized for the expression in mammalian cells the protective antigen sequence (of proteins S, S-del, S-Fc, RBD, RBD-G, RBD-Fc) of SARS-CoV-2 with a sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 by their sequential administration to mammals with a time interval of 1 week for the inducing specific immunity to SARS-CoV-2.

The experiment was performed according to the protocol described in example 7.

All the animals were divided into 29 groups (3 animals each,) injected intramuscularly with:

1. phosphate buffer (100 μ l), and a week later phosphate buffer (100 μ l)
2. phosphate buffer (100 μ l), and a week later Ad5-null 10^8 PFU/mouse
3. Ad5-null 10^8 PFU/mouse, and a week later phosphate buffer (100 μ l)
4. Ad5-null 10^8 PFU/mouse, and a week later Ad5-null 10^8 PFU/mouse
5. Ad5-S-CoV-2 10^8 PFU/mouse, and a week later Ad5-S-CoV-2 10^8 PFU/mouse
6. Ad5-S-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-CoV-2 10^8 PFU/mouse
7. Ad5-S-CoV-2 10^8 PFU/mouse, and a week later Ad5-S-del-CoV-2 10^8 PFU/mouse
8. Ad5-S-CoV-2 10^8 PFU/mouse, and a week later Ad5-S-Fc-CoV-2 10^8 PFU/mouse
9. Ad5-S-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-CoV-2 10^8 PFU/mouse
10. Ad5-S-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-G-CoV-2 10^8 PFU/mouse

11. Ad5-S-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-Fc-CoV-2 10^8 PFU/mouse
12. Ad5- S-del -CoV-2 10^8 PFU/mouse, and a week later Ad5-S-CoV-2 10^8 PFU/mouse
13. Ad5-S-del-CoV-2 10^8 PFU/mouse, and a week later Ad5-S-del-CoV-2 10^8 PFU/mouse
14. Ad5- S-del -CoV-2 10^8 PFU/mouse, and a week later Ad5-S-Fc-CoV-2 10^8 PFU/mouse
15. Ad5- S-del -CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-CoV-2 10^8 PFU/mouse
16. Ad5- S-del -CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-G-CoV-2 10^8
PFU/mouse
17. Ad5- S-del -CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-Fc-CoV-2
 10^8 PFU/mouse
18. Ad5- S-Fc -CoV-2 10^8 PFU/mouse, and a week later Ad5-S-CoV-2 10^8 PFU/mouse
19. Ad5- S-Fc -CoV-2 10^8 PFU/mouse, and a week later Ad5-S-del-CoV-2 10^8 PFU/mouse
20. Ad5- S-Fc -CoV-2 10^8 PFU/mouse, and a week later Ad5-S-Fc-CoV-2 10^8 PFU/mouse
21. Ad5- S-Fc -CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-CoV-2 10^8 PFU/mouse
22. Ad5- S-Fc -CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-G-CoV-2 10^8 PFU/mouse
23. Ad5- S-Fc -CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-Fc-CoV-2 10^8
PFU/mouse
24. Ad5-RBD-CoV-2 10^8 PFU/mouse, and a week later Ad5-S-CoV-2 10^8 PFU/mouse
25. Ad5-RBD-CoV-2 10^8 PFU/mouse, and a week later Ad5-S-del-CoV-2 10^8 PFU/mouse
26. Ad5-RBD-CoV-2 10^8 PFU/mouse, and a week later Ad5-S-Fc-CoV-2 10^8 PFU/mouse
27. Ad5-RBD-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-CoV-2 10^8 PFU/mouse
28. Ad5-RBD-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-G-CoV-2 10^8 PFU/mouse
29. Ad5-RBD-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-Fc-CoV-2 10^8 PFU/mouse
30. Ad5-RBD-G-CoV-2 10^8 PFU/mouse, and a week later Ad5-S-CoV-2 10^8 PFU/mouse
31. Ad5-RBD-G-CoV-2 10^8 PFU/mouse, and a week later Ad5-S-del-CoV-2 10^8 PFU/mouse
32. Ad5-RBD-G-CoV-2 10^8 PFU/mouse, and a week later Ad5-S-Fc-CoV-2 10^8 PFU/mouse
33. Ad5-RBD-G-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-CoV-2 10^8 PFU/mouse
34. Ad5-RBD-G-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-G-CoV-2
 10^8 PFU/mouse
35. Ad5-RBD-G-CoV-2 10^8 PFU/mouse, and a week later Ad5- RBD-Fc-CoV-2 10^8
PFU/mouse
36. Ad5-RBD-Fc-CoV-2 10^8 PFU/mouse, and a week later Ad5-S-CoV-2 10^8 PFU/mouse
37. Ad5-RBD-Fc-CoV-2 10^8 PFU/mouse, and a week later Ad5-S-del-CoV-2 10^8
PFU/mouse
38. Ad5-RBD-Fc-CoV-2 10^8 PFU/mouse and a week later Ad5-S-Fc-CoV-2 10^8 PFU/mouse

39. Ad5-RBD-Fc-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-CoV-2 10^8 PFU/mouse
40. Ad5-RBD-Fc-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-G-CoV-2 10^8 PFU/mouse
41. Ad5-RBD-Fc-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-Fc-CoV-2 10^8 PFU/mouse

The results are presented in Tables 4 and 5.

Table 4. – Antibody titers against the SARS-CoV-2 virus S protein in the blood serum of mice from control groups

Table 4.

		Second immunization (a week later)	
		PBS	Ad5-null
First immunization	PBS	0	0
	Ad5-null	0	0

Table 5. – Antibody titers against the SARS-CoV-2 virus S protein in the blood serum of mice from experimental groups

Table 5.

		Second immunization (a week later)					
First immunization		Ad5-S-CoV-2	Ad5-S-del-CoV-2	Ad5-S-Fc-CoV-2	Ad5-RBD-CoV-2	Ad5-RBD-G-CoV-2	Ad5-RBD-Fc-CoV-2
	Ad5-S-CoV-2	1:32768	1:131072	1:104032	1:131072	1:65536	1:104032
	Ad5-S-del-CoV-2	1:65536	1:131072	1:131072	1:131072	1:65536	1:104032
	Ad5-S-Fc-CoV-2	1:65536	1:104032	1:65536	1:104032	1:52016	1:131072

	Ad5-RBD-CoV-2	1:52016	1:65536	1:131072	1:65536	1:32768	1:52016
	Ad5-RBD-G-CoV-2	1:13107 2	1:131072	1:104032	1:131072	1:65536	1:104032
	Ad5-RBD-Fc-CoV-2	1:82570	1:131072	1:65536	1:32768	1:65536	1:65536

Thus, the experimental results have fully confirmed that the sequential immunization with the developed immunobiological agents in different combinations that include different forms of S protein of SARS-CoV-2 will cause a more powerful induction of immune response than the immunization with the one antigen performed according to a similar regimen.

Example 9. Effectiveness assessment of the immunization with the developed immunobiological agent by the percentage of proliferating lymphocytes.

Lymphocyte proliferation assay enables to assess the ability of lymphocytes to divide more actively after encountering an antigen. In order to assess proliferation, the authors used fluorescent dye CFSE for staining lymphocytes. This dye binds to cellular proteins and stays there for a long time, but it never spreads to the neighboring cells in the population. However, the fluorescent label is passed onto the daughter cells. The label concentration and, consequently, the fluorescence intensity in the daughter cells is decreased precisely twice. Thus, dividing cells can be easily traced by a decrease in their fluorescence. Therefore dividing cells can be easily traced by the reducing fluorescence intensity.

C57BL/6 mice were used in the experiment. All the animals were divided into 8 groups (3 animals each,) and injected intramuscularly with:

- 1) Phosphate buffer (100 μ l)
- 2) Ad5-null 10^8 PFU/mouse
- 3) Ad5-S-CoV-2 10^8 PFU/mouse
- 4) Ad5-S-del-CoV-2 10^8 PFU/mouse
- 5) Ad5-S-Fc-CoV-2 10^8 PFU/mouse
- 6) Ad5-RBD-CoV-2 10^8 PFU/mouse
- 7) Ad5-RBD-G-CoV-2 10^8 PFU/mouse
- 8) Ad5-RBD-Fc-CoV-2 10^8 PFU/mouse

Two weeks later the animals were euthanized. Lymphocytes were isolated from the spleen by Ficoll-Urografin density gradient centrifugation. Then, the isolated cells were stained with CFSE according to the CFSE technique (Monitoring lymphocyte proliferation in vitro and in vivo with the intracellular fluorescent dye carboxy fluorescent diacetate succinimidyl ester/ Quah BJ, Warren HS, Parish CR Nat Protoc. 2007; 2(9), p:2049-2056) and were cultured in the presence of antigen. Then, the cells were analyzed using flow cytometry. The obtained results are shown in Fig. 1, 2, 3, 4. Thus, it could be concluded that the obtained adenoviral constructs induce antigen-specific immune response (both CD4+ and CD8+).

As shown by the experiment results (Fig. 1, 2, 3, 4), the immunobiological agents developed according to claim 1, claim 2, claim 3, claim 4, claim 5 effectively stimulate proliferation of lymphocytes in the dose used.

Example 10. A method of utilization of the developed immunobiological agents based on recombinant human adenoviruses serotypes 5 and 26 by their sequential administration to mammals for the induction of specific immunity to SARS-CoV-2.

The experiment was performed according to the protocol described in example 7. Combinations of immunobiological agents were selected on the basis of examples 7 and 8.

All the animals were divided into 31 group (3 animals each,) injected intramuscularly with:

1. phosphate buffer (100 мкл), and a week later phosphate buffer (100 μl)
2. Ad26-null 10^8 PFU/mouse, and a week later phosphate buffer (100 μl)
3. phosphate buffer (100 μl), and a week later Ad26-null 10^8 PFU/mouse
4. Ad26-null 10^8 PFU/mouse, and a week later Ad26-null 10^8 PFU/mouse
5. Ad5-null 10^8 PFU/mouse, and a week later phosphate buffer (100 μl)
6. phosphate buffer (100 μl), and a week later Ad5-null 10^8 PFU/mouse
7. Ad5-null 10^8 PFU/mouse, and a week later Ad5-null 10^8 PFU/mouse
8. Ad5-null 10^8 PFU/mouse, and a week later Ad26-null 10^8 PFU/mouse
9. Ad26-null 10^8 PFU/mouse, and a week later Ad5-null 10^8 PFU/mouse
10. Ad5-S-CoV-2 10^8 PFU/mouse, and a week later Ad26-RBD-G-CoV-2 10^8 PFU/mouse
11. Ad5-RBD-G-CoV-2 10^8 PFU/mouse, and a week later Ad26-S-CoV-2 10^8 PFU/mouse
12. Ad26-S-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-G-CoV-2 10^8 PFU/mouse
13. Ad26-RBD-G-CoV-2 10^8 PFU/mouse, and a week later Ad5-S-CoV-2 10^8 PFU/mouse
14. Ad5-S-del-CoV-2 10^8 PFU/mouse, and a week later Ad26-RBD-CoV-2 10^8 PFU/mouse

15. Ad26-S-G-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-CoV-2 10^8 PFU/mouse
16. Ad5-RBD-CoV-2 10^8 PFU/mouse, and a week later Ad26-S-del-CoV-2 10^8 PFU/mouse
17. Ad26-S-G-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-CoV-2 10^8 PFU/mouse
18. Ad26-RBD-CoV-2 10^8 PFU/mouse, and a week later Ad5- S-del -CoV-2 10^8 PFU/mouse
19. Ad5-RBD-CoV-2 10^8 PFU/mouse, and a week later Ad26- S-del -CoV-2 10^8 PFU/mouse
20. Ad5-S-del-CoV-2 10^8 PFU/mouse, and a week later Ad26-RBD-G-CoV-2 10^8 PFU/mouse
21. Ad5-RBD-G-CoV-2 10^8 PFU/mouse, and a week later Ad26- S-del -CoV-2 10^8 PFU/mouse
22. Ad26-S-del-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-G-CoV-2 10^8 PFU/mouse
23. Ad26-RBD-G-CoV-2 10^8 PFU/mouse, and a week later Ad5-S-G-CoV-2 10^8 PFU/mouse
24. Ad5-S-CoV-2 10^8 PFU/mouse, and a week later Ad26-RBD-CoV-2 10^8 PFU/mouse
25. Ad26-S-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-CoV-2 10^8 PFU/mouse
26. Ad5-RBD-CoV-2 10^8 PFU/mouse, and a week later Ad26-S-CoV-2 10^8 PFU/mouse
27. Ad26-RBD-CoV-2 10^8 PFU/mouse, and a week later Ad5-S-CoV-2 10^8 PFU/mouse
28. Ad5- S-del -CoV-2 10^8 PFU/mouse, and a week later Ad26-S-CoV-2 10^8 PFU/mouse
29. Ad26- S-del -CoV-2 10^8 PFU/mouse, and a week later Ad5-S-CoV-2 10^8 PFU/mouse
30. Ad5-S-CoV-2 10^8 PFU/mouse, and a week later Ad26- S-del -CoV-2 10^8 PFU/mouse
31. Ad26-S-CoV-2 10^8 PFU/mouse, and a week later Ad5- S-del -CoV-2 10^8 PFU/mouse

The results are presented in Tables 6 and 7.

Table 6. – Antibody titers against the SARS-CoV-2 virus S protein in the blood serum of mice from experimental groups

Table 6.

		Second immunization (a week later)		
		PBS	Ad5-null	Ad26-null
First immunization	PBS	0	0	0
	Ad5-null	0	0	0

	Ad26-null	0	0	0
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Table 7. – Antibody titers against the SARS-CoV-2 virus S protein in the blood serum of mice from experimental groups

Table 7.

Group of animals	Geometric mean of antibody titers
Ad5-S-CoV-2 10^8 PFU/mouse, and a week later Ad26-RBD-G 10^8 PFU/mouse	1:208064
Ad5-RBD-G-CoV-2 10^8 PFU/mouse, and a week later Ad26-S-CoV-2 10^8 PFU/mouse	1:1321123
Ad26-S-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-G-CoV-2 10^8 PFU/mouse	1:832255
Ad26-RBD-G-CoV-2 10^8 PFU/mouse, and a week later Ad5-S-CoV-2 10^8 PFU/mouse	1:1321123
Ad5- S-del -CoV-2 10^8 PFU/mouse, and a week later Ad26-RBD-CoV-2 10^8 PFU/mouse	1:165140
Ad26- S-del -CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD -CoV-2 10^8 PFU/mouse	1:104032
Ad5-RBD-CoV-2 10^8 PFU/mouse, and a week later Ad26- S-del -CoV-2 10^8 PFU/mouse	1:104032
Ad26- S-del -CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-CoV-2 10^8 PFU/mouse	1:52016
Ad26-RBD-CoV-2 10^8 PFU/mouse, and a week later Ad5- S-del -CoV-2 10^8 PFU/mouse	1:131072
Ad5-RBD-CoV-2 10^8 PFU/mouse, and a week later Ad26- S-del -CoV-2 10^8 PFU/mouse	1:104032
Ad5- S-del -CoV-2 10^8 PFU/mouse, and a	1:165140

week later Ad26-RBD-G-CoV-2 10 ⁸ PFU/mouse	
Ad5-RBD-G-CoV-2 10 ⁸ PFU/mouse, and a week later Ad26- S-del -CoV-2 10 ⁸ PFU/mouse	1:208064
Ad26- S-del -CoV-2 10 ⁸ PFU/mouse, and a week later Ad5-RBD-G-CoV-2 10 ⁸ PFU/mouse	1:660561
Ad26-RBD-G-CoV-2 10 ⁸ PFU/mouse, and a week later Ad5- S-del -CoV-2 10 ⁸ PFU/mouse	1:416128
Ad5-S-CoV-2 10 ⁸ PFU/mouse, and a week later Ad26-RBD-CoV-2 10 ⁸ PFU/mouse	1:208064
Ad26-S-CoV-2 10 ⁸ PFU/mouse, and a week later Ad5-RBD-CoV-2 10 ⁸ PFU/mouse	1:65536
Ad5-RBD-CoV-2 10 ⁸ PFU/mouse, and a week later Ad26-S-CoV-2 10 ⁸ PFU/mouse	1:131072
Ad26-RBD-CoV-2 10 ⁸ PFU/mouse, and a week later Ad5-S-CoV-2 10 ⁸ PFU/mouse	1:165140
Ad5- S-del -CoV-2 10 ⁸ PFU/mouse, and a week later Ad26-S-CoV-2 10 ⁸ PFU/mouse	1:208064
Ad26-S-G-CoV-2 10 ⁸ PFU/mouse, and a week later Ad5-S-CoV-2 10 ⁸ PFU/mouse	1:208064
Ad5-S-CoV-2 10 ⁸ PFU/mouse, and a week later Ad26- S-del -CoV-2 10 ⁸ PFU/mouse	1:165140
Ad26-S-CoV-2 10 ⁸ PFU/mouse, and a week later Ad5- S-del -CoV-2 10 ⁸ PFU/mouse	1:165140

Thus, the experimental results have fully confirmed that the sequential immunization with the developed immunobiological agents which include different adenoviral vectors (based on human adenovirus serotypes 5 and 26) will cause a more powerful induction of

immune response than the immunization with one vector performed according to a similar immunization regimen.

Example 11. Effectiveness assessment of the immunization with the developed immunobiological agent by IFN-gamma induction

This experiment was conducted to assess the effectiveness of immunization with the developed immunobiological agent based on recombinant adenovirus containing optimized for the expression in mammalian cells the protective antigen sequence (of proteins S, S-del, S-Fc, RBD, RBD-G, RBD-Fc) of the SARS-CoV-2 virus with a sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, as estimated by increase in IFN-gamma concentration in the medium after the splenocytes of C57/BL6 mice, immunized with the adenoviral constructs, were stimulated with the SARS-CoV-2 virus recombinant full-length S protein.

Mouse IFN gamma Platinum ELISA Kit (Affymetrix eBioscience, USA) was used to determine IFN-gamma level.

ELISA protocol. Plate wells were washed twice with normal strength washing buffer at an amount of 200 µl per well, and then 100 µl of reference solutions and 100 µl of sample diluent, as a negative control, were added. 50 µl of sample diluent were placed in each of the wells, and then 50 µl of samples (medium from the stimulated splenocytes) were added to every well. Solution of biotin-conjugated antibodies was prepared. For this purpose, the conjugate in an amount of 60 µl was diluted in 5.94 ml of an assay buffer. Then, 50 µl of biotin-conjugated antibodies solution were placed in each of the wells. The plate was covered with a lid and incubated at room temperature in shaker at 400 rpm for 2 hours. Next, solution of streptavidin conjugated with horseradish peroxidase was prepared. For this purpose, the conjugate in an amount of 60 µl was diluted in 5.94 ml of assay buffer. The plate wells were washed twice with normal strength washing buffer at an amount of 200 µl per well and 100 µl of streptavidin solution conjugated with horseradish peroxidase were added to each of the plate wells. The plate was incubated at room temperature in shaker at 400 rpm for 1 hour. Then, the plate wells were washed twice with normal strength washing buffer at an amount of 200 µl per well and 100 µl of TMB substrate were added to each of the plate wells, and the plate was incubated under darkness at room temperature for 10 minutes. Then 100 µl of stop solution was added to each of the plate wells. The optical density was measured using plate spectrophotometer (Multiskan FC, Thermo) at a wavelength of 450 nm.

The results of measurement of IFN-gamma production at Day 15 after the immunization of experimental animals with adenoviral constructs are presented graphically in Fig. 5 as an increase in IFN-gamma concentration (-fold), wherein the cells stimulated with the SARS-CoV-2 virus recombinant full-length S protein are compared with intact cells.

The study results have demonstrated that the administration of the obtained constructs to animals was followed by a high level of induction of IFN-gamma expression in the splenocytes stimulated with the SARS-CoV-2 virus recombinant S protein, suggesting that specific T-cell-mediated immune response was formed.

Example 12. A method of utilization of the developed immunobiological agents based on recombinant human adenoviruses serotype 5, containing optimized for the expression in mammalian cells the protective antigen sequence (of S proteins and RBD-G) of SARS-CoV-2 with a sequence selected from SEQ ID NO:1 and SEQ ID NO:5 by their simultaneous administration to mammals for the induction of specific immunity to SARS-CoV-2.

The experiment was performed according to the protocol described in example 7. Combination of immunobiological agents was selected on the basis of examples 8 and 11.

All the animals were divided into 17 groups (5 animals each), injected intramuscularly with:

1. phosphate buffer (100 μ k)
2. Ad5-null 10^5 viral particles/mouse
3. Ad5-null 10^6 viral particles/mouse
4. Ad5-null 10^7 viral particles/mouse
5. Ad5-null 10^8 viral particles/mouse
6. Ad5-null 10^9 viral particles/mouse
7. Ad5-null 10^{10} viral particles/mouse
8. Ad5-null $5 \cdot 10^{10}$ viral particles/mouse
9. Ad5-null 10^{11} viral particles/mouse
10. Ad5-S-CoV-2 + Ad5-RBD-G-CoV-2 10^5 viral particles/mouse
11. Ad5-S-CoV-2 + Ad5-RBD-G-CoV-2 10^6 viral particles/mouse
12. Ad5-S-CoV-2 + Ad5-RBD-G-CoV-2 10^7 viral particles/mouse
13. Ad5-S-CoV-2 + Ad5-RBD-G-CoV-2 10^8 viral particles/mouse
14. Ad5-S-CoV-2 + Ad5-RBD-G-CoV-2 10^9 viral particles/mouse

15. Ad5-S-CoV-2 + Ad5-RBD-G-CoV-2 10^{10} viral particles/mouse
16. Ad5-S-CoV-2 + Ad5-RBD-G-CoV-2 $5 \cdot 10^{10}$ viral particles/mouse
17. Ad5-S-CoV-2 + Ad5-RBD-G-CoV-2 10^{11} viral particles/mouse

The results are presented in Tables 8 and 9.

Table 8. – Antibody titers against the SARS-CoV-2 virus S protein in the blood serum of mice from experimental groups

Table 8.

Group of animals	Geometric mean of antibody titers to S protein of SARS-CoV-2
phosphate buffer	0
Ad5-null 10^5 viral particles/mouse	0
Ad5-null 10^6 viral particles/mouse	0
Ad5-null 10^7 viral particles/mouse	0
Ad5-null 10^8 viral particles/mouse	0
Ad5-null 10^9 viral particles/mouse	0
Ad5-null 10^{10} viral particles/mouse	0
Ad5-null $5 \cdot 10^{10}$ viral particles/mouse	0
Ad5-null 10^{11} viral particles/mouse	0

Table 9. – Antibody titers against the SARS-CoV-2 virus S protein in the blood serum of mice from experimental groups

Table 9.

Group of animals, viral particles/mouse	Geometric mean of antibody titers to S protein of SARS-CoV-2
Ad5-S-CoV-2+ Ad5-RBD-G-CoV-2 10^5	0
Ad5-S-CoV-2+ Ad5-RBD-G-CoV-2 10^6	1:14263
Ad5-S-CoV-2+ Ad5-RBD-G-CoV-2 10^7	1:99334

Ad5-S-CoV-2+ Ad5-RBD-G-CoV-2 10^8	1:131072
Ad5-S-CoV-2+ Ad5-RBD-G-CoV-2 10^9	1:172951
Ad5-S-CoV-2+ Ad5-RBD-G-CoV-2 10^{10}	1:301124
Ad5-S-CoV-2+Ad5-RBD-G-CoV-2 $5 \cdot 10^{10}$	1:345901
Ad5-S-CoV-2+ Ad5-RBD-G-CoV-2 10^{11}	1:524288

Thus, the results of experiment have fully confirmed that the simultaneous immunization with the developed immunobiological agents induces humoral immune response to SARS-CoV-2 glycoprotein over the dose range from 10^6 viral particles/mouse to 10^{11} viral particles/mouse. Thus, it is obvious that dose escalation will result in antibody titer increase in the blood of mammals till the toxic effect occurs.

Example 13. A method of utilization of the developed immunobiological agent by sequential administration to mammals at different time intervals in an effective amount for the induction of specific immunity to SARS-CoV-2.

This example describes a method of utilization of the developed immunobiological agent based on recombinant human adenoviruses serotype 5, containing optimized for the expression in mammalian cells the protective antigen sequence (of proteins S, S-del, RBD, RBD-G, RBD-Fc) of SARS-CoV-2 with a sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, by their sequential administration to mammals with a time interval of 1 week or with a time interval of 3 weeks for the induction of specific immunity to SARS-CoV-2.

The experiment was performed according to the protocol described in example 7.

All the animals were divided into 28 groups (3 animals each), injected intramuscularly with:

1. phosphate buffer (100 μ l), and a week later phosphate buffer (100 μ l)
2. Ad5-null 10^8 PFU/mouse, and a week later Ad5-null 10^8 PFU/mouse
3. Ad5-S-CoV-2 10^8 PFU/mouse, and a week later Ad5-S-CoV-2 10^8 PFU/mouse

4. Ad5-S-del-CoV-2 10^8 PFU/mouse, and a week later Ad5-S-del-CoV-2 10^8 PFU/mouse
5. Ad5-S-Fc-CoV-2 10^8 PFU/mouse, and a week later Ad5-S-Fc-CoV-2 10^8 PFU/mouse
6. Ad5-RBD-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-CoV-2 10^8 PFU/mouse
7. Ad5-RBD-G-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-G-CoV-2 10^8 PFU/mouse
8. Ad5-RBD-Fc-CoV-2 10^8 PFU/mouse, and a week later Ad5-RBD-Fc-CoV-2 10^8 PFU/mouse
9. Ad26-S-CoV-2 10^8 PFU/mouse, and a week later Ad26-S-CoV-2 10^8 PFU/mouse
10. Ad26-S-del-CoV-2 10^8 PFU/mouse, and a week later Ad26-S-del-CoV-2 10^8 PFU/mouse
11. Ad26-S-Fc-CoV-2 10^8 PFU/mouse, and a week later Ad26-S-Fc-CoV-2 10^8 PFU/mouse
12. Ad26-RBD-CoV-2 10^8 PFU/mouse, and a week later Ad26-RBD-CoV-2 10^8 PFU/mouse
13. Ad26-RBD-G-CoV-2 10^8 PFU/mouse, and a week later Ad26-RBD-G-CoV-2 10^8 PFU/mouse
14. Ad26-RBD-Fc-CoV-2 10^8 PFU/mouse, and a week later Ad26-RBD-Fc-CoV-2 10^8 PFU/mouse
15. phosphate buffer (100 μ l), and 3 weeks later phosphate buffer (100 μ l)
16. Ad5-null 10^8 PFU/mouse, and 3 weeks later Ad5-null 10^8 PFU/mouse
17. Ad5-S-CoV-2 10^8 PFU/mouse, and 3 weeks later Ad5-S-CoV-2 10^8 PFU/mouse
18. Ad5-S-del-CoV-2 10^8 PFU/mouse and 3 weeks later Ad5-S-del-CoV-2 10^8 PFU/mouse
19. Ad5-S-Fc-CoV-2 10^8 PFU/mouse, and 3 weeks later Ad5-S-Fc-CoV-2 10^8 PFU/mouse
20. Ad5-RBD-CoV-2 10^8 PFU/mouse, and 3 weeks later Ad5-RBD-CoV-2 10^8 PFU/mouse
21. Ad5-RBD-G-CoV-2 10^8 PFU/mouse, and 3 weeks later Ad5-RBD-G-CoV-2 10^8 PFU/mouse
22. Ad5-RBD-Fc-CoV-2 10^8 PFU/mouse, and 3 weeks later Ad5-RBD-Fc-CoV-2 10^8 PFU/mouse
23. Ad26-S-CoV-2 10^8 PFU/mouse, and 3 weeks later Ad26-S-CoV-2 10^8 PFU/mouse
24. Ad26-S-del-CoV-2 10^8 PFU/mouse, and 3 weeks later Ad26-S-del-CoV-2 10^8 PFU/mouse
25. Ad26-S-Fc-CoV-2 10^8 PFU/mouse, and 3 weeks later Ad26-S-Fc-CoV-2 10^8 PFU/mouse
26. Ad26-RBD-CoV-2 10^8 PFU/mouse, and 3 weeks later Ad26-RBD-CoV-2 10^8 PFU/mouse
27. Ad26-RBD-G-CoV-2 10^8 PFU/mouse, and 3 weeks later Ad26-RBD-G-CoV-2 10^8 PFU/mouse
28. Ad26-RBD-Fc-CoV-2 10^8 PFU/mouse, and 3 weeks later Ad26-RBD-Fc-CoV-2 10^8 PFU/mouse

The results are presented in Table 10.

Table 10. – Antibody titers against the SARS-CoV-2 virus S protein in the blood serum of mice

Table 10.

	Second immunization	
	with a time interval of 1 week	with a time interval of 2 weeks
PBS/PBS	0	0
Ad5-null/ Ad5-null	0	0
Ad5-S-CoV-2/ Ad5-S-CoV-2	1:32768	1:41285
Ad5-S-del-CoV-2/ Ad5-S-del-CoV-2	1:41285	1:52016
Ad5-S-Fc-CoV-2/ Ad5-S-Fc-CoV-2	1:82570	1:104032
Ad5-RBD-CoV-2/ Ad5-RBD-CoV-2	1:65536	1:82570
Ad5-RBD-G-CoV-2/ Ad5-RBD-G-CoV-2	1:65536	1:82570
Ad5-RBD-Fc-CoV-2/ Ad5-RBD-Fc-CoV-2	1:65536	1:104032
Ad26-S-CoV-2/ Ad26-S-CoV-2	1:26008	1:32768
Ad26-S-del-CoV-2/ Ad26-S-del-CoV-2	1:52016	1:65536
Ad26-S-Fc-CoV-2/ Ad26-S-Fc-CoV-2	1:26008	1:52016
Ad26-RBD-CoV-2/ Ad26-RBD-CoV-2	1:20643	1:26008
Ad26-RBD-G-CoV-2/	1:41285	1:52016

Ad26-RBD-G-CoV-2		
Ad26-RBD-Fc-CoV-2/ Ad26-RBD-Fc-CoV-2	1:13004	1:16384

Thus, the results of the experiment prove that the sequential immunization with the developed immunobiological agent generates higher immune response levels than a single immunization. For an average-level specialist it appears obvious that the final regimen of immunization with finished product is based on multi-year studies and frequently adjusted by physician, depending various factors, such as the target group of patients, their age, epidemiological situation, etc.

Example 14.

A method of utilization of the developed immunobiological agent by the sequential administration to mammals with a time interval of one week in an effective amount for the induction of specific immunity to SARS-CoV-2.

This example describes a method of utilization of the developed immunobiological agent based on recombinant human adenoviruses serotype 5 and recombinant human adenoviruses serotype 26, by their sequential administration to mammals with a time interval of 1 week for the induction of specific immunity to SARS-CoV-2.

The experiment was performed according to the protocol described in example 7.

All the animals were divided into 9 groups (5 animals each,), injected intramuscularly with:

1. phosphate buffer (100 μ l), and then a week later phosphate buffer (100 μ l), and then a week later phosphate buffer (100 μ l)
2. Ad5-null 10^8 PFU/mouse, and then a week later Ad5-null 10^8 PFU/mouse, and then a week later Ad5-null 10^8 PFU/mouse
3. Ad26-null 10^8 PFU/mouse, and then a week later Ad26-null 10^8 PFU/mouse, and then a week later Ad26-null 10^8 PFU/mouse

4. Ad5-S-CoV-2 10^8 PFU/mouse, and then a week later Ad5-S-CoV-2 10^8 PFU/mouse, and then a week later Ad5-S-CoV-2 10^8 PFU/mouse
5. Ad5-S-CoV-2 10^8 PFU/mouse, and then a week later Ad26-S-CoV-2 10^8 PFU/mouse, and then a week later Ad5-S-CoV-2 10^8 PFU/mouse
6. Ad5-S-CoV-2 10^8 PFU/mouse, and then a week later Ad26-S-CoV-2 10^8 PFU/mouse, and then a week later Ad26-S-CoV-2 10^8 PFU/mouse
7. Ad26-S-CoV-2 10^8 PFU/mouse, and then a week later Ad26-S-CoV-2 10^8 PFU/mouse, and then a week later Ad26-S-CoV-2 10^8 PFU/mouse
8. Ad26-S-CoV-2 10^8 PFU/mouse, and then a week later Ad5-S-CoV-2 10^8 PFU/mouse, and then a week later Ad26-S-CoV-2 10^8 PFU/mouse
9. Ad26-S-CoV-2 10^8 PFU/mouse, and then a week later Ad5-S-CoV-2 10^8 PFU/mouse, and then a week later Ad5-S-CoV-2 10^8 PFU/mouse

The results are presented in Table 11.

Table 11. – Antibody titers against the SARS-CoV-2 virus S protein in the blood serum of mice

Table 11.

Group of animals	Antibody titer
PBS/ PBS / PBS	0
Ad5-null/ Ad5-null/ Ad5-null	0
Ad26-null/ Ad26-null/ Ad26-null	0
Ad5-S-CoV-2/ Ad5-S-CoV-2/ Ad5-S-CoV-2	1:150562
Ad5-S-CoV-2/ Ad26-S-CoV-2/ Ad5-S-CoV-2	1:301124
Ad5-S-CoV-2/ Ad26-S-CoV-2/ Ad26-S-CoV-2	1:228209
Ad26-S-CoV-2/ Ad26-S-CoV-2/ Ad26-S-CoV-2	1:172950
Ad26-S-CoV-2/ Ad5-S-CoV-2/ Ad26-S-CoV-2	1:262144
Ad26-S-CoV-2/ Ad5-S-CoV-2/ Ad5-S-CoV-2	1:301124

Thus, the results of this experiment with the developed immunobiological agent based on recombinant human adenoviruses serotype 5 or serotype 26, containing the SARS-CoV-2 virus S protein sequence, optimized for the expression in mammalian cells, have demonstrated that the sequential three-times administration of any variant of this agent will

cause a more powerful induction of immune response to the antigen than when its administered once or twice. For an average-level specialist it appears obvious that the developed immunobiological agent can be administered according to a multiple-dose schedule that will cause antibody titer increase in the blood of mammals up to the level when the toxic effect occurs. The required number of immunizations may vary, depending on the target population category (nationality, age, occupation, etc.). The frequency of immunization is also dependent on a cost-benefit assessment.

Example 15.

A method of utilization of the developed immunobiological agent administered once to mammals by different routes in an effective amount for the induction of specific immunity to SARS-CoV-2.

This example describes a method of utilization of the developed immunobiological agent based on recombinant human adenoviruses serotype 5 and recombinant human adenoviruses serotype 26, administered once to mammals by 3 routes (intranasal, subcutaneous, intramuscular) for the induction of specific immunity to SARS-CoV-2.

The experiment was performed according to the protocol described in example 7.

All the animals were divided into 15 groups (3 animals each,), injected with:

1. PBS intranasally
2. PBS subcutaneously
3. PBS intramuscularly
4. Ad5-null 10^9 PFU/mouse intranasally
5. Ad5-null 10^9 PFU/mouse subcutaneously
6. Ad5-null 10^9 PFU/mouse intramuscularly
7. Ad26-null 10^9 PFU/mouse intranasally
8. Ad26-null 10^9 PFU/mouse subcutaneously
9. Ad26-null 10^9 PFU/mouse intramuscularly
10. Ad5-S-CoV-2 10^9 PFU/mouse intranasally
11. Ad5-S-CoV-2 10^9 PFU/mouse subcutaneously

12. Ad5-S-CoV-2 10^9 PFU/mouse intramuscularly
13. Ad26-S-CoV-2 10^9 PFU/mouse intranasally
14. Ad26-S-CoV-2 10^9 PFU/mouse subcutaneously
15. Ad26-S-CoV-2 10^9 PFU/mouse intramuscularly

The results are presented in Table 12.

Table 12. – Antibody titers against the SARS-CoV-2 virus S protein in the blood serum of mice

Table 12.

Group of animals	Antibody titer
PBS intranasally	0
PBS subcutaneously	0
PBS intramuscularly	0
Ad5-null intranasally	0
Ad5-null subcutaneously	0
Ad5-null intramuscularly	0
Ad26-null intranasally	0
Ad26-null subcutaneously	0
Ad26-null intramuscularly	0
Ad5-S-CoV-2 intranasally	1:16384
Ad5-S-CoV-2 subcutaneously	1:26008
Ad5-S-CoV-2 intramuscularly	1:57052
Ad26-S-CoV-2 intranasally	1:13004
Ad26-S-CoV-2 subcutaneously	1:24300
Ad26-S-CoV-2 intramuscularly	1:43238

Thus, the results of this experiment confirm the possibility of utilization of the developed immunobiological agent for inducing specific immunity to the SARS-CoV-2 virus by its intranasal, intramuscular or subcutaneous route of administration.

Industrial Applicability

The advantage of claimed technical solution is a utilization of such doses of recombinant adenoviruses, expressing the full-length protein gene, that allow enhancing immunogenicity, but not yet causing toxic effects in animals. An additional increase in immunogenicity of the receptor-binding domain of the SARS-CoV-2 virus S gene, as a result of linking the leader sequence for facilitating protein secretion from the cell to the environment, could be also considered as an advantage. The presence of adequate T-cell-mediated response (both CD4+, and CD8+) to the administered antigen is a further advantage of claimed technical solution.

Thus, an immunobiological agent has been created which is based on recombinant human adenoviruses serotype 5, containing human adenoviruses serotype 5 with deleted E1/E3 sites, and an integrated genetic construct, encoding the developed optimal amino acid sequences of the SARS-CoV-2 virus S protective antigen.

Also, an immunobiological agent has been created which is based on recombinant human adenoviruses serotype 26, containing human adenoviruses serotype 26 with deleted E1/E3 sites and an integrated genetic construct, encoding the developed optimal amino acid sequences of the SARS-CoV-2 virus S protective antigen. The expression of encoding sequences of different types of the SARS-CoV-2 virus S protein is ensured by recombinant pseudo-adenoviral particles in the subject's body.

The developed immunobiological agent could be considered for use in pre-clinical trials as an antiviral vaccine, capable to provide effective human protection against infection caused by the SARS-CoV-2 coronavirus. Technology of production of such vaccine is claimed.

Claims

1. Immunobiological agent for the prevention of diseases caused by severe acute respiratory syndrome virus SARS-CoV-2 based on recombinant human adenovirus serotype 5 or recombinant human adenovirus serotype 26, containing optimized for the expression in mammalian cells the sequence of S protective antigen of the SARS-CoV-2 virus with gene C'-terminal deletion of 18 amino acids (SEQ ID NO:2).
2. Immunobiological agent for the prevention of diseases caused by severe acute respiratory syndrome virus SARS-CoV-2 based on recombinant human adenovirus serotype 5 or recombinant human adenovirus serotype 26, containing optimized for the expression in mammalian cells the sequence of full-length S protective antigen of the SARS-CoV-2 virus and the human IgG1 Fc-fragment sequence (SEQ ID NO:3)
3. Immunobiological agent for the prevention of diseases caused by severe acute respiratory syndrome virus SARS-CoV-2 based on recombinant human adenovirus serotype 5 or recombinant human adenovirus serotype 26, containing optimized for the expression in mammalian cells the SARS-CoV-2 virus S protein receptor-binding domain sequence with the viral leader peptide sequence (SEQ ID NO:4).
4. Immunobiological agent for the prevention of diseases caused by severe acute respiratory syndrome virus SARS-CoV-2 based on recombinant human adenovirus serotype 5 or recombinant human adenovirus serotype 26, containing optimized for the expression in mammalian cells the SARS-CoV-2 virus protein S receptor-binding domain sequence with the transmembrane domain of vesicular stomatitis virus glycoprotein (SEQ ID NO:5)
5. Immunobiological agent for the prevention of diseases caused by the severe acute respiratory syndrome (SARS-CoV-2) virus based on recombinant human adenovirus serotype 5, or recombinant human adenovirus serotype 26, containing optimized for the expression in mammalian cells the SARS-CoV-2 virus S protein receptor-binding domain sequence with the leader peptide sequence and the human IgG1 Fc-fragment sequence (SEQ ID NO:6).
6. Immunobiological agent for the prevention of diseases caused by the severe acute respiratory syndrome (SARS-CoV-2) virus based on recombinant human adenovirus serotype 5, or recombinant human adenovirus serotype 26, containing optimized for the expression in mammalian cells the SARS-CoV-2 virus full-length S protective antigen sequence on the basis of sequences of S protein genes of the SARS-CoV-2 virus (SEQ ID NO:1) in combination with immunobiological agents presented herein in claim 1 and/or claim 2, and/or claim 3, and/or claim 4, and/or claim 5, and/or claim 6.

7. Method of induction of specific immunity to the SARS-CoV-2 virus, involving the administration to mammals of one or more agents presented herein in claim 1 and/or claim 2, and/or claim 3, and/or claim 4, and/or claim 5, and/or claim 6, in an effective amount.
8. Method presented herein in claim 7, wherein two different immunobiological agents based on recombinant human adenovirus serotype 5 or two different immunobiological agents based on recombinant human adenovirus serotype 26 presented herein in claim 1 and/or claim 2, and/or claim 3, and/or claim 4, and/or claim 5, and/or claim 6, are administered to mammals with a time interval of more than one week.
9. Method presented herein in claim 7, wherein any of the immunobiological agents based on recombinant human adenovirus serotype 5 and any of the immunobiological agents based on recombinant human adenovirus serotype 26 presented herein in claim 1 and/or claim 2, and/or claim 3, and/or claim 4, and/or claim 5, and/or claim 6, are sequentially administered to mammals with a time interval of more than one week, or any of the immunobiological agents based on recombinant human adenovirus serotype 26 and any of the immunobiological agents based on recombinant human adenovirus serotype 5 presented herein in claim 1 and/or claim 2, and/or claim 3, and/or claim 4, and/or claim 5, and/or claim 6, are sequentially administered to mammals with a time interval of more than one week.
10. Method presented herein in claim 7, wherein any two immunobiological agents based on recombinant human adenovirus serotype 5 or serotype 26 presented herein in claim 1 and/or claim 2, and/or claim 3, and/or claim 4, and/or claim 5, and/or claim 6, are simultaneously administered to mammals.

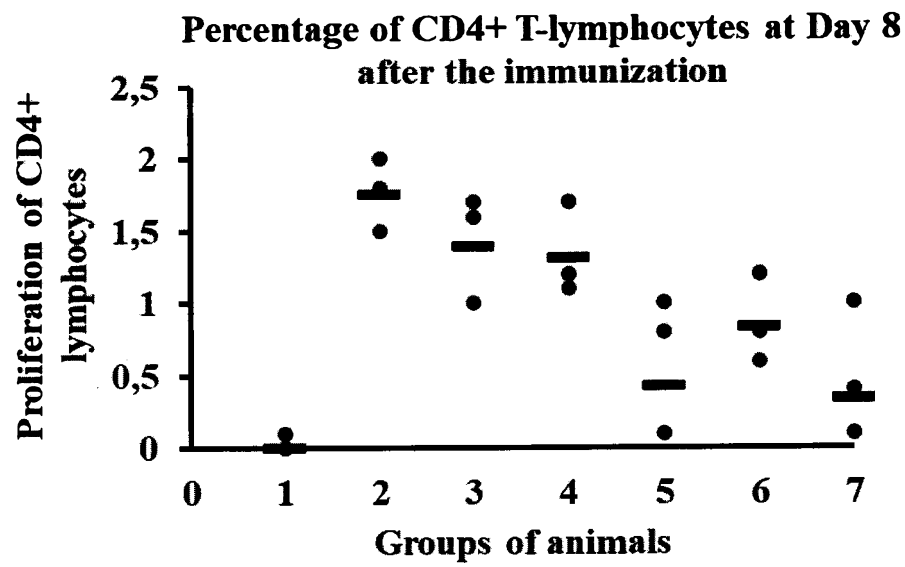


FIG. 1

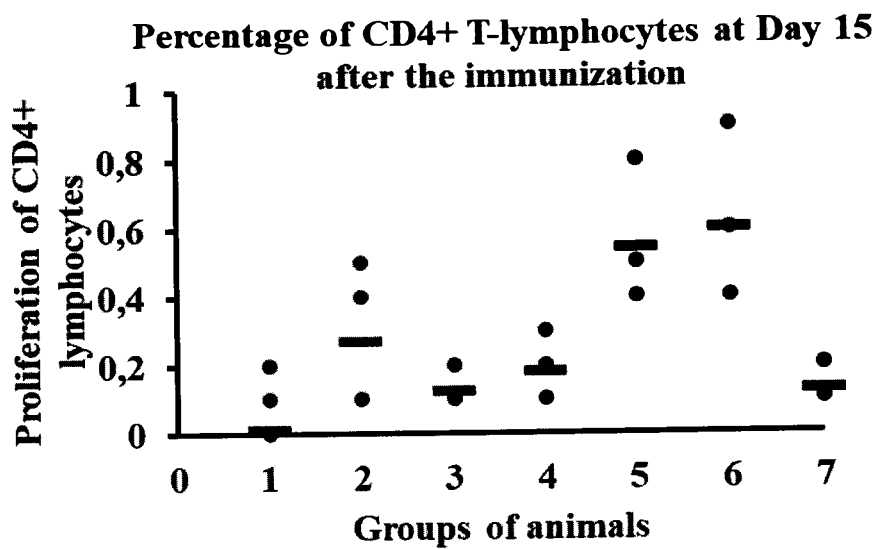


FIG. 2

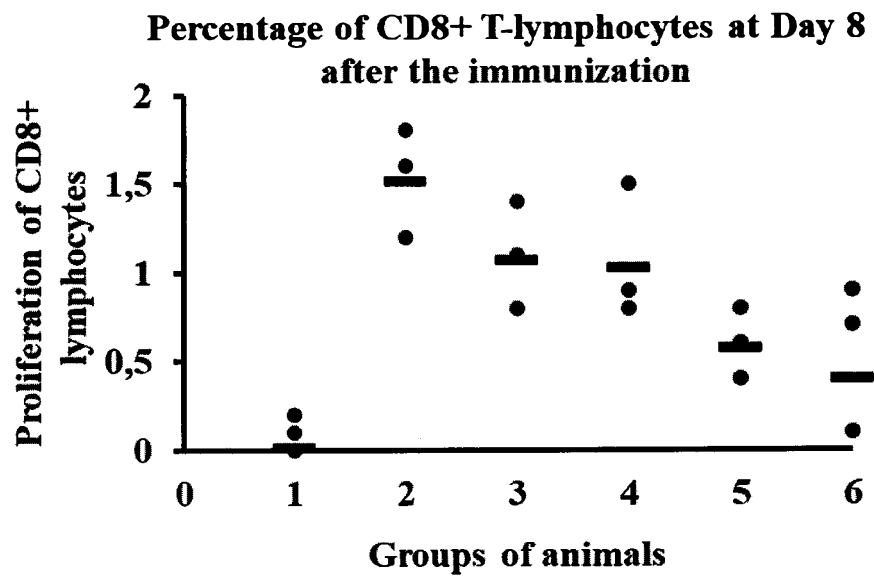


FIG. 3

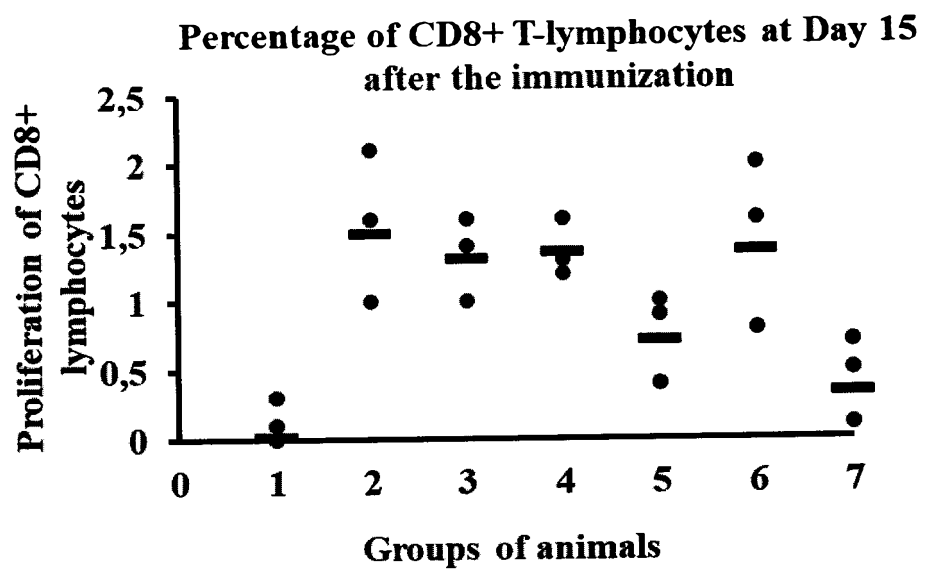


FIG. 4

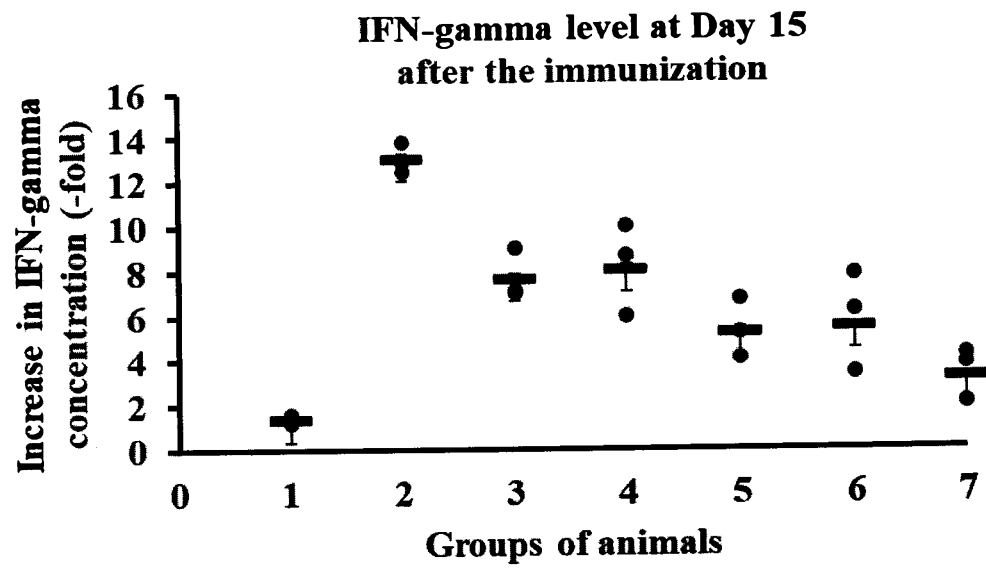


FIG. 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/RU 2020/000344

A. CLASSIFICATION OF SUBJECT MATTER A61K 39/215 (2006.01) A61P 31/12 (2006.01)		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
A61K, A61P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
PatSearch, EMBL, NCBI, Espacenet, DWPI, USPTO, CIPO (Canada PO), SIPO		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KR20200032050 A (KIM SEUNG CHAN), 25.03.2020, abstract, claims, [0005]	1-10
A	US2008267992 A1 (CANCER CT SUN YAT SUN UNIVERSI), 30.10.2008, abstract, claims, [0014], [0026]	1-10
A	LIANG MF et al., SARS patients-derived human recombinant antibodies to S and M proteins efficiently neutralize SARS-coronavirus infectivity, Biomed Environ Sci., 2005, 18(6), 363-374, abstract	1-10
A	NCBI Reference Sequence: YP_009724390.1, 30.03.2020 [online] {retrived on 10.09.2020}. Retrived from <NCBI Database>	1-10
A	LANYING DU et al., A 219-mer CHO-Expressing Receptor-Binding Domain of SARS-CoV S Protein Induces Potent Immune Responses and Protective Immunity, Viral Immunol., 2010, Vol. 23, N.2, pp 211–219, abstact	1-10
☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.		
* Special categories of cited documents:	“A” document defining the general state of the art which is not considered to be of particular relevance “D” document cited by the applicant in the international application “E” earlier document but published on or after the international filing date “L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) “O” document referring to an oral disclosure, use, exhibition or other means “P” document published prior to the international filing date but later than the priority date claimed “T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention “X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone “Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art “&” document member of the same patent family	
Date of the actual completion of the international search	Date of mailing of the international search report	
10 September 2020 (10.09.2020)	24 September 2020 (24.09.2020)	
Name and mailing address of the ISA/RU: Federal Institute of Industrial Property, Berezhkovskaya nab., 30-1, Moscow, G-59, GSP-3, Russia, 125993 Facsimile No: (8-495) 531-63-18, (8-499) 243-33-37	Authorized officer E. Tereshkina Telephone No. (8-499) 240-25-91	