



(51) International Patent Classification:

A61K 39/215 (2006.01) C12N 7/00 (2006.01)
A61P 31/14 (2006.01) C12N 15/86 (2006.01)

(21) International Application Number:

PCT/RU2020/000589

(22) International Filing Date:

06 November 2020 (06.11.2020)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

2020127979 22 August 2020 (22.08.2020) RU

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,

(54) Title: EXPRESSION VECTOR AGAINST SEVERE ACUTE RESPIRATORY SYNDROME VIRUS SARS-COV-2

(57) Abstract: The invention relates to biotechnology, immunology and virology. There is created an expression vector containing the genome of recombinant human adenovirus serotype 26, wherein the E1 and E3 regions are deleted, and the ORF6-Ad26 region is replaced by ORF6-Ad5, with an integrated expression cassette selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 (variant 1). Therein, the sequence SEQ ID NO: 5 was used as a parental sequence of human adenovirus serotype 26. Further, there is created an expression vector, containing the genome of recombinant simian adenovirus serotype 25, wherein the E1 and E3 regions are deleted, with an integrated expression cassette selected from SEQ ID NO: 4, SEQ ID NO: 2, SEQ ID NO: 3 (variant 2). Therein, the sequence SEQ ID NO: 6 was used as a parental sequence of simian adenovirus serotype 25. Furthermore, there is created an there is created containing the genome of recombinant human adenovirus serotype 5, wherein the E1 and E3 regions are deleted, with an integrated expression cassette selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 (variant 3). Therein, the sequence SEQ ID NO: 7 was used as a parental sequence of human adenovirus serotype 5. There is also developed a method of utilization of the developed expression vector for creating an immunobiological agent for the induction of specific immunity against severe acute respiratory syndrome virus SARS-CoV-2.

TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
- *upon request of the applicant, before the expiration of the time limit referred to in Article 21(2)(a)*
- *with sequence listing part of description (Rule 5.2(a))*

EXPRESSION VECTOR AGAINST SEVERE ACUTE RESPIRATORY SYNDROME VIRUS SARS-COV-2

Field of the Invention

The invention relates to biotechnology, immunology and virology. It covers recombinant vectors that can be used in pharmaceutical industry to develop an immunobiological agent for inducing specific immunity against severe acute respiratory syndrome virus SARS-CoV-2.

Background of the Invention

In December 2019, a disease caused by a novel coronavirus (SARS-CoV-2) was found in Wuhan, the provincial capital of Hubei. The disease posed complex tasks to be handled by public health experts and medical doctors, including rapid diagnostic methods and clinical management of patients. The SARS-CoV-2 virus has spread fast around the globe and progressed into a pandemic of an unprecedented scale. By August 19, 2020 the number of cases was more than 22 million and the number of deaths – 791 thousand.

So far, only limited data are available about epidemiology, clinical signs, prevention and treatment of this disease. As known, pneumonia is the most common clinical manifestation of the infection caused by a novel coronavirus, and the development of acute respiratory distress syndrome (ARDS) is reported in a considerable number of patients. The virus is assigned to Group II of dangerous pathogens likewise other viruses of the same family (SARS-CoV and MERS-CoV). Currently, no agents for specific prevention or etiotropic treatment of the novel coronavirus disease are available.

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

One of the promising areas in vaccinology is focused on the development of viral vector-based agents for the prevention of diseases. In this context, human adenovirus serotype 5-based systems are the most widely used tools in the pharmaceutical industry.

This type of vectors has advantages such as a high safety, capability to enter different cell types, high packaging capacity, the possibility to derive products with high titers, etc.

There is a solution (CN1276777C) which suggests using a vaccine against severe acute respiratory syndrome based on recombinant human adenovirus serotype 5 containing the SARS-CoV virus S protein sequence.

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.

There is a solution according to CN111218459 which describes the development of an expression vector based on human adenovirus serotype 5 with the deleted E1 and E3 regions, containing S protein gene. This vector is used for designing vaccine against COVID-19.

At the same time, a broad application of the vectors based on human adenovirus serotype 5 is limited, as some people have pre-existing immune response. Thus, the focus turns to the development of multiple vectors with genetic variations, e.g. those based on adenoviruses of other serotypes

Implementation of the Invention

The technical aim of the claimed group of inventions is to induce a sustained immune response to SARS-CoV-2 glycoprotein and to ensure the presence of biologically effective protective antibody titer against SARS-CoV-2 glycoprotein. It will enable to create an immunobiological agent for inducing specific immunity against severe acute respiratory syndrome virus SARS-CoV-2.

The technical result is the creation of an expression vector containing a genome of recombinant human adenovirus serotype 26, wherein the E1 and E3 regions are deleted and the ORF6-Ad26 region is replaced by ORF6-Ad5, with a placed expression cassette selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 (variant 1). With that, the sequence SEQ ID NO:5 was used as a parental sequence of human adenovirus serotype 26.

Further, the technical result is the creation of an expression vector containing a genome of recombinant simian adenovirus serotype 25, wherein the E1 and E3 regions are deleted, with a placed expression cassette selected from SEQ ID NO:4, SEQ ID NO:2, SEQ ID NO:3 (variant 2). With that, the sequence SEQ ID NO:6 was used as a parental sequence of simian adenovirus serotype 25.

Furthermore, the technical result is the creation of an expression vector containing a genome of recombinant human adenovirus serotype 5, wherein the E1 and E3 regions are deleted, with a placed expression cassette selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 (variant 3). With that, the sequence SEQ ID NO:7 was used as a parental sequence of human adenovirus serotype 5.

This technical result is also achieved by that there is developed a method of utilization of the developed expression vector for the creation of an immunobiological agent for inducing specific immunity against severe acute respiratory syndrome virus SARS-CoV-2.

Embodiment of the Invention

The method of obtaining an expression vector containing the genome of recombinant human adenovirus serotype 26 is that at the first stage there is constructed a plasmid comprising two homologous regions of the genome of human adenovirus serotype 26, which is then linearized, using restriction endonuclease, and mixed with the DNA isolated from the virions of human adenovirus serotype 26, and homologous recombination is conducted in E.coli cells. As a result, there is received a plasmid carrying the genome of recombinant human adenovirus serotype 26 with the deleted E1 region. Next, using the genetic engineering methods, an open reading frame 6 (ORF6) is replaced by ORF6 of human adenovirus serotype 5. Then, the E3 region is deleted in order to expand packaging capacity. Ultimately, the expression cassette is inserted into the vector.

The method of obtaining an expression vector containing the genome of recombinant simian adenovirus serotype 25 is as follows: at the first stage, there is constructed a plasmid comprising two homologous regions of the genome of simian adenovirus serotype 25, which is then linearized using restriction endonuclease and mixed with the DNA isolated from the virions of simian adenovirus serotype 25, and homologous recombination is conducted in E.coli cells. As a result, there is received a plasmid carrying the genome of simian adenovirus serotype 25 with the deleted E1 region. Then, the E3 region is deleted in order to expand packaging capacity. Ultimately, the expression cassette is inserted into the vector.

The method of obtaining an expression vector containing the genome of recombinant human adenovirus serotype 5 is as follows: at the first stage, there is constructed a plasmid comprising two homologous regions of the genome of human adenovirus serotype 5, which is then linearized using restriction endonuclease and mixed with the DNA isolated from the virions of human adenovirus serotype 5, and homologous recombination is conducted in E.coli cells. As a result, there is received a plasmid carrying the genome of human adenovirus serotype 5 with

the deleted E1 region. Next, using the genetic engineering methods, the E3 region is deleted in order to expand packaging capacity. Ultimately, the expression cassette is inserted into the vector.

To maximize the effectiveness of induction of immune reactions, the authors claimed multiple variants of expression cassettes.

Spike (S) protein of the SARS-CoV-2 virus optimized for the expression in mammalian cells was used as an antigen in all cassettes. The S protein is one of the coronavirus structural proteins. It is exposed on the viral particle surface and is responsible for binding to ACE2 (angiotensin-converting enzyme 2) receptor. The results of completed studies demonstrated the production of virus-neutralizing antibodies to the S protein, and therefore it is considered as a promising antigen for the development of pharmaceutical agents.

The expression cassette SEQ ID NO:1 contains the CMV promoter, SARS-CoV-2 virus S protein gene, and polyadenylation signal.

The expression cassette SEQ ID NO:2 contains the CAG promoter, SARS-CoV-2 virus S protein gene, and polyadenylation signal.

The expression cassette SEQ ID NO:3 contains the EF1 promoter, SARS-CoV-2 virus S protein gene, and polyadenylation signal.

The expression cassette SEQ ID NO:4 contains the CMV promoter, SARS-CoV-2 virus S protein gene, and polyadenylation signal.

To confirm the effectiveness of this invention, there was assessed a capability of the developed expression vectors to induce immune response in animals against severe acute respiratory syndrome virus SARS-CoV-2.

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

Example 1

Production of an expression vector containing the genome of recombinant human adenovirus serotype 26.

At the first stage, a plasmid construction pAd26-Ends was designed which carries two regions homologous to the genome of human adenovirus serotype 26 (two homology arms) and the ampicillin-resistance gene. One of the homology arms is the beginning portion of the genome of human adenovirus serotype 26 (from the left inverted terminal repeat to the E1 region) and sequence of the viral genome including pIX protein. The other homology arm contains a

nucleotide sequence localized after ORF3 E4 region through the end of the genome. Synthesis of pAd26-Ends construction was performed by the Moscow company “Eurogen” ZAO.

The human adenovirus serotype 26 DNA isolated from the virions was mixed with pAd26-Ends. A plasmid pAd26-dlE1, carrying the genome of human adenovirus serotype 26 with the deleted E1 region, was obtained through the process of homologous recombination between pAd26-Ends and the viral DNA.

Then, in the obtained plasmid pAd26-dlE1, using routine cloning techniques, the sequence containing an open reading frame 6 (ORF6-Ad26) was replaced with a similar sequence from the genome of human adenovirus serotype 5 in order to ensure that human adenovirus serotype 26 is capable to replicate effectively in HEK293 cell culture. As a result, the plasmid pAd26-dlE1-ORF6-Ad5 was derived.

Further, using routine genetic engineering techniques, the E3 region (approx. 3321 base pairs between the genes pVIII and U-exon) of the adenoviral genome was deleted from the constructed plasmid pAd26-dlE1-ORF6-Ad5 in order to expand packaging capacity of the vector. Ultimately, a recombinant vector pAd26-only-null based on the genome of human adenovirus serotype 26 with the open reading frame ORF6 of human adenovirus serotype 5 and with the deleted E1 and E3 regions was obtained. The sequence SEQ ID NO:5 was used as a parental sequence of human adenovirus serotype 26.

Also, the authors developed multiple designs of the expression cassette:

- the expression cassette SEQ ID NO:1 contains the CMV promoter, SARS-CoV-2 virus S protein gene, and polyadenylation signal.
- the expression cassette SEQ ID NO:2 contains the CAG promoter, SARS-CoV-2 virus S protein gene, and polyadenylation signal.
- the expression cassette SEQ ID NO:3 contains the EF1 promoter, SARS-CoV-2 virus S protein gene, and polyadenylation signal.

Based on the plasmid construction pAd26-Ends, using genetic engineering techniques, there were obtained constructions pArms-26-CMV-S-CoV2, pArms-26-CAG-S-CoV2, pArms-26-EF1-S-CoV2, containing the expression cassettes SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, respectively, as well as the carrying homology arms of the genome of adenovirus serotype 26. Next, the constructions pArms-26-CMV-S-CoV2, pArms-26-CAG-S-CoV2, pArms-26-EF1-S-CoV2 were linearized by a unique hydrolysis site between the homology arms; each of the plasmids was mixed with the recombinant vector pAd26-only-null. The homologous

recombination allowed obtaining the plasmids pAd26-only-CMV-S-CoV2, pAd26-only-CAG-S-CoV2, pAd26-only-EF1-S-CoV2 which carry the genome of recombinant human adenovirus serotype 26 with the open reading frame ORF6 of human adenovirus serotype 5 and the deletion of E1 and E3 regions, with the expression cassette SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, respectively.

During the fourth stage, the plasmids pAd26-only-CMV-S-CoV2, pAd26-only-CAG-S-CoV2, pAd26-only-EF1-S-CoV2 were hydrolyzed with the specific restriction endonucleases to remove the vector part. The derived DNA products were used for the transfection of HEK293 cell culture.

Thus, there was obtained an expression vector containing the genome of recombinant human adenovirus serotype 26, wherein the E1 and E3 regions are deleted and the RF6-Ad26 region is replaced by ORF6-Ad5, with an integrated expression cassette selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3.

Example 2

Production of an expression vector, containing the genome of recombinant simian adenovirus serotype 25.

At the first stage, a plasmid construction pSim25-Ends was designed which carries two regions homologous to the genome of simian adenovirus serotype 25 (two homology arms). One of the homology arms is the beginning portion of the genome of simian adenovirus serotype 25 (from the left inverted terminal repeat to the E1 region) and sequence from the end of the E1-region to the pIVa2 protein. The other homology arm contains a sequence of the end portion of the adenoviral genome, including the right inverted terminal repeat. Synthesis of the pSim25-Ends construction was performed by the Moscow company "Eurogen" ZAO.

The simian adenovirus serotype 25 DNA isolated from the virions was mixed with pSim25-Ends. A plasmid pSim25-dIE1, carrying the genome of simian adenovirus serotype 25 with the deleted E1 region, was obtained through the process of homologous recombination between pSim25-Ends and the viral DNA.

Further, using routine genetic engineering techniques, the E3 region of the adenoviral genome (approx. 3921base pairs from the beginning portion of gene 12,5K to gene 14.7K) was deleted from the constructed plasmid pSim25-dIE1 in order to expand packaging capacity of the vector. Ultimately, there was obtained a plasmid construction pSim25-null, encoding a full-

length genome of simian adenovirus serotype 25 with the deleted E1 and E3 regions. The sequence SEQ ID NO:6 was used as a parental sequence of simian adenovirus serotype 25.

Also, the authors developed multiple designs of the expression cassette:

- the expression cassette SEQ ID NO:4 contains the CMV promoter, SARS-CoV-2 virus S protein gene, and polyadenylation signal.
- the expression cassette SEQ ID NO:2 contains the CAG promoter, SARS-CoV-2 virus S protein gene, and polyadenylation signal.
- the expression cassette SEQ ID NO:3 contains the EF1 promoter, SARS-CoV-2 virus S protein gene, and polyadenylation signal.

Then, based on the plasmid construction pSim25-Ends, using genetic engineering techniques, there were obtained constructions pArms-Sim25-CMV-S-CoV2, pArms-Sim25-CAG-S-CoV2, pArms-Sim25-EF1-S-CoV2, containing the expression cassettes SEQ ID NO:4, SEQ ID NO:2, or SEQ ID NO:3, respectively, as well as the carrying homology arms from the genome of simian adenovirus serotype 25. Next, the constructions pArms-Sim25-CMV-S-CoV2, pArms-Sim25-CAG-S-CoV2, pArms-Sim25-EF1-S-CoV2 were linearized by a unique hydrolysis site between the homology arms; each of the plasmids was mixed with the recombinant vector pSim25-null. As a result of homologous recombination there were obtained the recombinant plasmid vectors pSim25-CMV-S-CoV2, pSim25-CAG-S-CoV2, pSim25-EF1-S-CoV2, containing a full-length genome of simian adenovirus serotype 25 with the deleted E1 and E3 regions, and the expression cassette SEQ ID NO:4, SEQ ID NO:2, or SEQ ID NO:3, respectively.

During the third stage, the plasmids pSim25-CMV-S-CoV2, pSim25-CAG-S-CoV2, pSim25-EF1-S-CoV2 were hydrolyzed with the specific restriction endonuclease to remove the vector part. The derived DNA products were used for the transfection of HEK293 cell culture. The produced material was used for generating preparative amounts of the recombinant adenoviruses.

As a result, recombinant human adenoviruses serotype 25 were obtained which contain SARS-CoV-2 virus S protein gene: simAd25-CMV-S-CoV2 (containing the expression cassette SEQ ID NO:4); simAd25-CAG-S-CoV2 (containing the expression cassette SEQ ID NO:2); simAd25-EF1-S-CoV2 (containing the expression cassette SEQ ID NO:3).

Thus, an expression vector was obtained which contains the genome of recombinant simian adenovirus 25, wherein the E1 and E3 regions are deleted, with an integrated expression cassette selected from SEQ ID NO:4, SEQ ID NO:2, SEQ ID NO:3.

Example 3

Production of an expression vector containing the genome of recombinant human adenovirus serotype 5.

At the first stage, a plasmid construction pAd5-Ends was designed which carries two regions homologous to the genome of human adenovirus serotype 5 (two homology arms). One of the homology arms is the beginning portion of the genome of human adenovirus serotype 5 (from the left inverted terminal repeat to the E1 region) and sequence of the viral genome including pIX protein. The other homology arm contains a nucleotide sequence after the E4-region ORF3 through the end of the genome. Synthesis of pAd5-Ends construction was performed by the Moscow company "Eurogen" ZAO.

The human adenovirus serotype 5 DNA isolated from the virions was mixed with pAd5-Ends. A plasmid pAd5-dlE1, carrying the genome of human adenovirus serotype 5 with the deleted E1 region, was obtained through the homologous recombination between pAd5-Ends and the viral DNA.

Further, using routine genetic engineering techniques, the E3 region of the adenoviral genome (2685 base pairs from the end of gene 12,5K to the beginning of sequence of U-exon) was deleted from the constructed plasmid pAd5-dlE1 in order to expand packaging capacity of the vector. Ultimately, there was obtained a recombinant plasmid vector pAd5-too-null, based on the genome of human adenovirus serotype 5 with the deleted E1 and E3 regions of the genome. The sequence SEQ ID NO:7 was used as a parental sequence of human adenovirus serotype 5.

Also, the authors developed multiple designs of the expression cassette:

- the expression cassette SEQ ID NO:1 contains the CMV promoter, SARS-CoV-2 virus S protein gene, and polyadenylation signal.
- the expression cassette SEQ ID NO:2 contains the CAG promoter, SARS-CoV-2 virus S protein gene, and polyadenylation signal.
- the expression cassette SEQ ID NO:3 contains the EF1 promoter, SARS-CoV-2 virus S protein gene, and polyadenylation signal.

Then, based on the plasmid construction pAd5-Ends, using genetic engineering techniques, there were obtained constructions pArms-Ad5-CMV-S-CoV2, pArms-Ad5-CAG-S-CoV2, pArms-Ad5-EF1-S-CoV2, containing the expression cassettes SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, respectively, as well as the carrying homology arms from the genome of human adenovirus serotype 5.

Next, the constructions pArms-Ad5-CMV-S-CoV2, pArms-Ad5-CAG-S-CoV2, pArms-Ad5-EF1-S-CoV2 were linearized by a unique hydrolysis site between the homology arms; each of the plasmids was mixed with the recombinant vector pAd5-too-null. As a result of homologous recombination there were obtained the plasmids pAd5-too-CMV-S-CoV2, pAd5-too-GAC-S-CoV2, pAd5-too-EF1-S-CoV2, carrying the genome of recombinant human adenovirus serotype 5 with the deleted the E1 and E3 regions, and the expression cassettes SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, respectively.

During the fourth stage, the plasmids pAd5-too-CMV-S-CoV2, pAd5-too-GAC-S-CoV2, pAd5-too-EF1-S-CoV2 were hydrolyzed with the specific restriction endonuclease to remove the vector part. The derived DNA product was used for the transfection of HEK293 cell culture. The produced material was used for generating preparative amounts of the recombinant adenovirus.

As a result, recombinant human adenoviruses serotype 5 were obtained which contain SARS-CoV-2 virus S protein gene: Ad5-CMV-S-CoV2 (containing the expression cassette SEQ ID NO:1); Ad5-CAG-S-CoV2 (containing the expression cassette SEQ ID NO:2); Ad5-EF1-S-CoV2 (containing the expression cassette SEQ ID NO:3).

Thus, an expression vector was obtained which contains the genome of recombinant human adenovirus serotype 5, wherein the E1 and E3 regions are deleted, with an integrated expression cassette selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3.

Example 4

Verification of the expression of SARS-CoV-2 virus S protein gene by the developed expression vectors in HEK293 cells.

The aim of this experiment was to verify the ability of constructed recombinant adenoviruses to express severe acute respiratory syndrome SARS-CoV-2 virus S protein gene in mammalian cells.

HEK293 cells were cultured in DMEM medium with supplemented 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 the expression vectors were added, one at a time. Thus, the following groups were formed:

- 1) Ad26-CMV-S-CoV2;
- 2) Ad26- CAG -S-CoV2;
- 3) Ad26- EF1-S-CoV2;
- 4) Ad26- null;
- 5) simAd25-CMV-S-CoV2;
- 6) simAd25- CAG -S-CoV2;
- 7) simAd25- EF1-S-CoV2;
- 8) simAd25- null;
- 9) Ad5-CMV-S-CoV2;
- 10) Ad5- CAG -S-CoV2;
- 11) Ad5- EF1-S-CoV2;
- 12) Ad5- null;
- 13) phosphate buffered saline.

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.

Next, 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 1.

Table 1 – Results of the experiment for verifying the expression of SARS-CoV-2 virus S protein gene in HEK293 cells after the addition of the developed expression vectors

Mean optical density at a wavelength of 450 nm

	Mean optical density at a wavelength of 450 nm
Ad26-CMV-S-CoV2	1.65 ($\pm$ 0.21)
Ad26- CAG -S-CoV2	1.61 ($\pm$ 0.15)
Ad26- EF1-S-CoV2	1.69 ($\pm$ 0.19)
Ad26- null	0.22 ($\pm$ 0.09)
simAd25-CMV-S-CoV2	1.70 ($\pm$ 0.20)
simAd25- CAG -S-CoV2	1.64 ($\pm$ 0.17)
simAd25- EF1-S-CoV2	1.65 ($\pm$ 0.14)
simAd25- null	0.19 ($\pm$ 0.08)
Ad5-CMV-S-CoV2	1.69 ($\pm$ 0.15)
Ad5- CAG -S-CoV2	1.68 ($\pm$ 0.17)
Ad5- EF1-S-CoV2	1.64 ($\pm$ 0.15)
Ad5- null	0.15 ($\pm$ 0.04)
phosphate buffered saline	0.17 ($\pm$ 0.08)

As shown by the received data, the expression of the target S protein of SARS-CoV-2 was observed in all cells transduced with the developed expression vectors.

Example 5

Assessment of the effectiveness of animal immunization with the developed expression vectors

One of the main characteristics of immunization effectiveness is an antibody titer. Example presents data relating to changes in the antibody titer against SARS-CoV-2 glycoprotein at day 21 after immunization

The mammalian species – BALB/c mice, females weighing 18 g were used in the experiment. All animals were divided into 13 groups, 5 animals per group, to whom the developed expression vector was injected intramuscularly at a dose 10^8 viral particles/100 μ l. Thus, the following groups of animals were formed:

- 1) Ad26-CMV-S-CoV2;
- 2) Ad26- CAG -S-CoV2;
- 3) Ad26- EF1-S-CoV2;
- 4) Ad26- null;
- 5) simAd25-CMV-S-CoV2;
- 6) simAd25- CAG -S-CoV2;
- 7) simAd25- EF1-S-CoV2;
- 8) simAd25- null;
- 9) Ad5-CMV-S-CoV2;
- 10) Ad5- CAG -S-CoV2;
- 11) Ad5- EF1-S-CoV2;
- 12) Ad5- null;
- 13) phosphate buffered saline.

Three weeks later, blood samples were taken from the tail vein of the animals, and blood serum was separated. An enzyme-linked immunosorbent assay (ELISA) was used to measure antibody titers according to the following protocol:

- 1) Protein (S) was adsorbed onto wells of a 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 µl 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) Further, secondary antibodies against mouse immunoglobulins conjugated with horseradish peroxidase were added.
- 8) Next, 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 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 1.

Table 1 - Antibody titer against S protein in the blood serum of mice (geometric mean of antibody titer)

Table 1

No	Designation of animal group	Antibody titer
1	Ad26-CMV-S-CoV2	14,703
2	Ad26- CAG -S-CoV2	12,800
3	Ad26- EF1-S-CoV2	16,890
4	Ad26- null	0
5	simAd25-CMV-S-CoV2	12,800
6	simAd25- CAG -S-CoV2	10,159
7	simAd25- EF1-S-CoV2	12,800
8	simAd25- null	0
9	Ad5-CMV-S-CoV2	11,143
10	Ad5- CAG -S-CoV2	16,127
11	Ad5- EF1-S-CoV2	12,800
12	Ad5- null	0

13	phosphate buffered saline	0
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As shown in the presented data, all the developed expression vectors induce sustained immune response to SARS-CoV-2 glycoprotein, as well as the presence of biologically effective protective antibody titer to SARS-CoV-2 glycoprotein. Thus, they can be used for creating an immunobiological agent for the induction of specific immunity against severe acute respiratory syndrome virus SARS-CoV-2

Thereby, the assigned technical aim, in particular, the induction of sustained immune response to SARS-CoV-2 glycoprotein as well as the presence of biologically effective protective antibody titer to SARS-CoV-2 glycoprotein is accomplished as proven by the provided examples.

Industrial Applicability

All the provided examples confirm the effectiveness of the expression vectors, their applicability for creating an immunobiological agent for the induction of specific immunity against severe acute respiratory syndrome virus SARS-CoV-2 and the industrial applicability.

Claims

1. Expression vector containing the genome of recombinant human adenovirus serotype 26, wherein the E1 and E3 regions are deleted, and the ORF6-Ad26 region is replaced by ORF6-Ad5, with an integrated expression cassette selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3.
2. Expression vector presented herein in claim 1 being distinct in that the SEQ ID NO:5 was used as a parental sequence of human adenovirus serotype 26.
3. Expression vector containing the genome of recombinant simian adenovirus serotype 25, wherein the E1 and E3 regions are deleted, with an integrated expression cassette selected from SEQ ID NO:4, SEQ ID NO:2, SEQ ID NO:3.
4. Expression vector presented herein in claim 3 being distinct in that the SEQ ID NO:6 was used as a parental sequence of simian adenovirus serotype 25.
5. Expression vector containing the genome of recombinant human adenovirus serotype 5, wherein the E1 and E3 regions are deleted, with an integrated expression cassette selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3.
6. Expression vector presented herein in claim 5 being distinct in that the SEQ ID NO:7 was used as a parental sequence of human adenovirus serotype 5.
7. Utilization of the expression vector presented herein in claims 1-6 for creating an immunobiological agent for the induction of specific immunity against severe acute respiratory syndrome virus SARS-CoV-2.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/RU 2020/000589

A. CLASSIFICATION OF SUBJECT MATTER		
<i>A61K 39/215 (2006.01)</i> <i>A61P 31/14 (2006.01)</i> <i>C12N 7/00 (2006.01)</i> <i>C12N 15/86 (2006.01)</i>		
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 39/215, A61P 31/14, C12N 7/00, 15/86		
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 (RUPTO Internal), USPTO, PAJ, Espacenet, Information Retrieval System of FIPS		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KR 102050616 B1 (JANSSEN VACCINES & PREVENTION B.V.) 03.12.2019	1-7
A	WO 2000/012740 A2 (DUKE UNIVERSITY et al.) 09.03.2000	1-7
A	US 2019/0134178 A1 (ALTIMMUNE INC.) 09.05.2019	1-7
A	WO 2010037027 A2 (AUBURN UNIVERSITY et al.) 01.04.2010	1-7
A	US 6251677 B1 (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA) 26.06.2001	1-7
☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.		
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"P" document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search	Date of mailing of the international search report	
16 December 2020 (16.12.2020)	16 December 2020 (16.12.2020)	
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