Immunogenic properties of SARS-CoV-2 inactivated by ultraviolet light

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Abstract
Vaccination against COVID-19 is the most effective method of controlling the spread of SARS-CoV-2 and reducing mortality from this disease. The development of vaccines with high protective activity against a wide range of SARS-CoV-2 antigenic variants remains relevant. In this regard, evaluation of the effectiveness of physical methods of virus inactivation, such as ultraviolet irradiation (UV) of the virus stock, remains relevant. This study demonstrates that the UV treatment of SARS-CoV-2 completely inactivates its infectivity while preserving its morphology, antigenic properties, and ability to induce the production of virus-neutralizing antibodies in mice through immunization. Thus, the UV inactivation of SARS-CoV-2 makes it possible to obtain viral material similar in its antigenic and immunogenic properties to the native antigen, which can be used both for the development of diagnostic test systems and for the development of an inactivated vaccine against COVID-19.

Introduction
Vaccination against COVID-19 is the most effective method of controlling the spread of SARS-CoV-2 and reducing mortality from this disease. Vaccines based on viral vectors, self-replicating RNA, and recombinant and native viral antigens are widely used worldwide [1–5]. Despite unprecedented preventive measures and the widespread use of vaccines against COVID-19, the pandemic spread of the SARS-CoV-2 coronavirus continues even in countries with high vaccination coverage [6]. Several countries, including Russia, are affected by severe epidemiological conditions and high morbidity and mortality rates [6]. New strains of SARS-CoV-2, differing from the original Wuhan strain in their antigenic and biological properties, are reported regularly [7–9]. Thus, from August to November 2021, the genetic variant Delta B.1.617.2 of SARS-CoV-2, which replaced the Alpha, Beta, and Gamma variants, occupied at least 95% of the global incidence structure (https://www.gisaid.org/) [10]. The Delta variant has increased infectivity and is less efficiently neutralized by antisera obtained from recovered COVID-19 patients who had been infected with other variants [11–13]. Furthermore, the SARS-CoV-2 variant Omicron B.1.1.529 has high epidemiological significance and is classified by WHO as a variant of concern (VOC). The Omicron genome has several deletions and more than 30 amino acid substitutions in the S protein, resulting in increased binding affinity of the virus for the ACE-2 receptor and, consequently, increased transmissibility and ability to evade neutralizing antibodies [14]. Thus, research on the development of vaccines with high protective activity against a wide range of SARS-CoV-2 antigenic variants remains relevant.

According to WHO, 137 candidate COVID-19 vaccines are licensed or in clinical trials, while 194 candidates are in preclinical trials as of 14.01.2022 [15]. Among the 132
vaccine candidates in various stages of clinical trials, 13% are inactivated-virus-based vaccines. The development of whole-virion inactivated vaccines is of particular interest, since such vaccines include the full set of structural viral proteins. The assurance of complete inactivation of the virus coupled with retaining the native conformation of the protective antigens is one of the most important requirements for whole-virion vaccines. Inactivated vaccines against COVID-19 are mainly produced by chemical methods based on the treatment of viral stock with β-propiolactone [5, 16–19] and/or formaldehyde [20]. Chemical inactivation can cause modifications and cross-linking of viral proteins, leading to conformational changes in viral antigens [21]. Furthermore, if toxic inactivating agents are used, additional steps are required to purify the viral antigen [16].

In this regard, evaluation of the effectiveness of physical methods of virus inactivation, such as ultraviolet irradiation of the virus stock, remains relevant. The aim of this work was to evaluate the effect of the SARS-CoV-2 virus inactivation with ultraviolet light (UV) on its morphology and antigenic and immunogenic properties. To achieve this goal, a preparation of UV-inactivated SARS-CoV-2 was obtained and investigated by immunochemical and virological methods.

**Materials and methods**

**Virus and cells**

Specimens of SARS-CoV-2 strains isolated in Vero cells in the Moscow region (Russia) belonging to different lineages, including the variants of concern Delta and Omicron, were used in the study (Table 1). Strain Dubrovka (GenBank ID: MW514307.1) [22], which is phylogenetically related to the Wuhan-Hu-1 strain (GenBank ID: NC_045512.2), was used for evaluation of immunogenic properties of UV-inactivated virus. All of the viruses (Table 1) were isolated and characterized by the authors of this study.

The virus was cultivated on the African green monkey kidney epithelial cell line Vero CCL81 (ATCC) (hereinafter referred to as Vero cells). Vero cells were maintained at 37°C in Earl-buffer-based DMEM medium (PanEco, Russia) in 5% fetal bovine serum (FBS) (Gibco, USA), with L-glutamine (PanEco) (300 µg/ml) and gentamicin (PanEco) (40 µg/ml) in an atmosphere of 5% CO₂. The neutralization reaction was performed using Earl-buffer-based DMEM nutrient medium supplemented with 1% FBS, L-glutamine (300 µg/ml), and gentamicin (40 µg/ml).

**Mice**

Female BALB/c mice, weighing 16-18 g (n = 25), obtained from the “Stezar” cattery (Russia), were used for immunization. The animals were kept in the animal facility of the I. I. Mechnikov Research Institute of Vaccines and Sera.

**Sera from convalescent COVID-19 patients**

Serum samples obtained from patients with a confirmed diagnosis of COVID-19 were provided by the Clinical Diagnostic Centre of the I. I. Mechnikov Research Institute of Vaccines and Sera. Work with clinical material was carried out under international ethical standards and with the consent of the patients.

**Virus cultivation**

A monolayer of Vero cells obtained within 72 hours of cultivation was infected with the Dubrovka strain of SARS-CoV-2 at different multiplicities of infection (MOI). Virus adsorption was carried out in a CO₂ incubator for 60 min, maintenance medium (DMEM, L-glutamine [300 µg/ml] and gentamicin [40 µg/ml]) was added, and the cells were incubated at 37°C in an atmosphere of 5% CO₂. To study the kinetics of virus reproduction, supernatants were collected every 12 hours for 4 days and stored at −80°C until tested by titration or quantitation by reverse-transcription real-time PCR.

**Virus titration**

SARS-CoV-2 titers were determined by the cytopathic effect endpoint method (CPE) in Vero cells. Four replicates of tenfold dilutions of the virus in maintenance medium were added to the wells of a 96-well plate and incubated for 5 days at 37°C in an atmosphere of 5% CO₂. The cell monolayer was inspected visually by microscopic examination for the

| Strain (isolate) | Collection date | GenBank ID of full genome sequence | Pangolin lineage | Variant of concern (WHO) | Passage level | Titer, log10 TCID₅₀/ml |
|------------------|-----------------|-----------------------------------|-----------------|--------------------------|--------------|----------------------|
| Dubrovka         | 2020-06-04      | MW514307.1                        | B.1.1.317       | -                        | 17           | 7.85                 |
| Altufjevo        | 2022-01-25      | ON032859.1                        | B.1.1.529.1.1   | Omicron                  | 5            | 5.7                  |
| Podolsk          | 2021-10-08      | ON032860.1                        | B.1.617.2.122   | Delta                    | 9            | 6.1                  |
presence of characteristic CPE at 120 hours postinfection (rounding of cells and detachment of cells from the monolayer). The virus titer was calculated as described by Ramakrishnan et al. [23] and expressed as log$_{10}$ TCID$_{50}$/ml.

**MTT assay**

The viability of virus-infected Vero cells was assessed by using the vital dye methyl thiazolyl tetrazolium (MTT) bromide. On day 5 postinfection, 20 μl of MTT solution (5 mg/ml; PanEco) was added to the cell-containing wells of the 96-well plate, which was incubated at 37°C in 5% CO$_2$ for 2 hours. Then, the medium was removed, and 100 μl of dimethyl sulfoxide (Sigma-Aldrich) was added to each well. Using a plate spectrophotometer, the OD of each well was measured at 530 nm, taking into account the baseline values at 620 nm. The viability of cells was calculated using the following formula:

% Viability = \( \frac{OD_{530 \text{ test sample}}}{OD_{530 \text{ cell control}}} \times 100 \)

where “OD$_{530 \text{ test sample}}$” is the mean OD$_{530}$ value in the wells with infected cells and “OD$_{530 \text{ cell control}}$” is the mean OD$_{530}$ value in the wells with uninfected cells.

**Quantification of SARS-CoV-2 RNA**

Viral RNA was isolated from culture samples using a MagnoPrime UNI reagent kit (NextBio, Russia) according to the manufacturer’s instructions. To detect viral RNA, we used primers and a probe designed based on the sequence of the nucleocapsid N gene of SARS-CoV-2 virus – CoVN-F, COVN-R, and COVN-P, respectively (Table 2) – described by Chan et al. [24]. A 2.5x Taq-polymerase reaction mixture reagent kit and MMLV reverse transcriptase (Syntol, Russia) were used to perform reverse transcription (RT)-PCR. The reaction mixture contained 10 pmol of each primer, 5 pmol of the probe, Taq DNA polymerase, and 30 units of reverse transcriptase. The amplification program was as follows: one cycle of 45°C for 10 min, one cycle of 95°C for 5 min, and 45 cycles of 95°C for 5 s and 55°C for 45 s. The reaction was performed in a DTprime thermocycler (DNA-Technology, Russia). All primers and probes were synthesized at Syntol. Samples obtained by successive tenfold dilutions of a known concentration of the synthetic oligonucleotide COVN-PC (Table 2) were used to construct a calibration curve.

**Inactivation of SARS-CoV-2 by ultraviolet (UV) light**

The supernatant of infected Vero cells was collected 72 hours after infection with the virus, clarified by centrifugation at 4000 rpm, and titrated. Virus inactivation (titer, 8.75 log$_{10}$ TCID$_{50}$/ml) was performed using a UV lamp (Philips TUV 30W/G30 T8, Holland). The length and diameter of the light tube were 895 mm and 28 mm, respectively, and the wavelength of the lamp was 253.7 nm. A Petri dish, 150 mm in diameter (177 cm$^2$), with 50 ml of viral material (5 mm thickness of the liquid layer), was placed under the lamp at a distance of 30 cm and irradiated for 0.5, 1, 2, 4, 6, 8, and 12 minutes with triple shaking of the liquid at equal time intervals (Fig. 1). Virus inactivation was monitored by three blind passages of the irradiated viral material on Vero cells, examining the cells for cytopathic effect and measuring the concentration of viral RNA in each of the passages.

![Fig. 1](https://biorender.com/about/)

**Table 2** Primeers, probes, and oligonucleotides used in this work

| Name    | Sequence (5’-3’)                  | Use                  | Source                     |
|---------|-----------------------------------|----------------------|----------------------------|
| CoVN-F  | GCGTCTTCTCGGAATGTGC              | Forward primer       | Chan et al. [24]           |
| COVN-R  | TTGGATCTCTTTTGCATCCAATTTC        | Reverse primer       |                            |
| COVN-P  | FAM-AACGTGGTGGACCTACAGGT-BHQ1    | Probe                |                            |
| COVN-PC | GCGTCTTCTCGGAATGTGCGCGCATTTGCAATGGATCCTACGTACAGGTGCGCATCA  |
|         | GAAACCTTCTCGGCAACCGGAACGTGGTGGTGCAACCTACACGTACAGGTGCGCATCA  |
|         | AATGGAAATGACCAAAGATCCAA          | Calibration          | This work                  |


The intensity of UV-C radiation was measured using a UV radiometer with a TKA-PKM (13) attenuating filter (NTP TKA LLC, Russia). For use in the ELISA and immunochromatography (IC) tests, 45 ml of UV-inactivated virus-containing supernatant (8.75 log10 TCID50/ml) was clarified by centrifugation and passed through a 100-kDa Amicon MWCO centrifuge filter (Millipore, Ireland) at 4000 rpm. The virus preparation collected on the filter was diluted to 4.5 ml with sterile PBS (pH 7.2), achieving a tenfold concentration of SARS-CoV-2 virions. Before use, the preparation was processed on an MSE ultrasonic disintegrator (UK) at an amplitude of 2 for 2 minutes.

**Evaluation of the antigenic properties of UV-inactivated SARS-CoV-2**

Fivefold dilutions of UV-inactivated SARS-CoV-2 were analyzed by ELISA and immunochromatography (IC), determining the highest dilution that gave a positive result. The SARS-CoV-2 antigen was detected in the IC reaction using a SARS-CoV-2 Rapid Antigen Test reagent kit (SD Biosensor Inc, Republic of Korea) according to the instructions. To determine the detection limit of SARS-CoV-2 antigen by ELISA, fivefold dilutions of UV-inactivated SARS-CoV-2 concentrated on 100-kDa Amicon MWCO columns (Millipore, Ireland) were coated onto 96-well immunoassay plates (Costar 2592 High-binding). ELISA was performed using sera from convalescent COVID-19 patients at dilution 1:200, using a BioKit ELISA reagent kit (Bioservis, Russia) according to the instructions. Horseradish-peroxidase-labeled murine monoclonal antibodies to the Fc fragment of human γ-globulin (Bioservis, Russia) at a dilution of 1:60,000 were used for detection.

**Immunization of mice**

To assess the immunogenicity of the inactivated virus, female BALB/c mice (n = 25) were divided into five groups of five animals and injected subcutaneously into the withers with 200 µL of the virus preparation twice with a 21-day interval according to the protocol shown in Table 3.

| Group of mice | 1 (n = 5) | 2 (n = 5) | 3 (n = 5) | 4 (n = 5) | 5 (n = 5) |
|---------------|-----------|-----------|-----------|-----------|-----------|
| First immunization | Virus-UV | Virus-UV +CFA | Virus-UV + Al(OH)3 | Infectious SARS-CoV-2 | PBS |
| Second immunization (21 days later) | Virus-UV | Virus-UV +IFA | Virus-UV + Al(OH)3 | - | PBS |

Viral material was mixed with adjuvants so that each mouse received a dose corresponding to 7.0 log10 TCID50. Mice in the control group were injected with PBS. On the day of the first immunization and 2 weeks after each immunization, blood samples were collected from the tail vein of the animals.

Immediately before immunization, viral material was mixed with an equal volume of Freund’s adjuvant (CFA or IFA) (Difco Laboratories, USA) and repeatedly (at least 15 times) passed through a fine injection needle. The emulsion was administered to the experimental animals within 10 min after preparation.

The viral material was adsorbed onto aluminum hydroxide (Sigma) so that a single injection dose (200 µL) contained 1.7 µg of adjuvant. The resulting mixture was incubated with regular shaking for 24 hours at +4°C before use.

**Antibody titration of mouse sera by ELISA**

Determination of antibody titers against SARS-CoV-2 in mouse sera was performed using a BioKit ELISA reagent kit (Bioservis, Russia) according to the instructions. Native viral antigen obtained as described above was coated at a dilution of 1:100 onto the wells of an immunoassay plate. Duplicate dilutions of the sera were analyzed by ELISA, starting at a dilution of 1:50. Horseradish-peroxidase-conjugated goat anti-mouse IgG, IgA, and IgM antibodies (IMTEC, Russia) were used in a dilution of 1:10,000 for detection of murine antibodies. The reciprocal value of the last dilution at which the OD value of the sample was higher than the cutoff for each assay was taken as the titer of SARS-CoV-2 antibodies. The OD value for the negative serum multiplied by 2 was used as the cutoff.

**Neutralization reaction**

Titers of SARS-CoV-2-neutralizing antibodies (NA) were determined as described by Gracheva et al. [22]. Frozen serum samples (100 µL) were thawed and heated at 56°C for 30 min, and twofold serial dilutions were prepared using maintenance medium. The serum dilutions were then mixed with an equal volume of a SARS-CoV-2 preparation containing 2 × 10^3 TCID50/ml and incubated at 37°C in a 5% CO2 atmosphere for 1 h. A 100-µL aliquot of the mixture of virus and serum was then added to a three-day monolayer...
Immunogenic properties of UV-inactivated SARS-CoV-2

Immunogenic properties of UV-inactivated SARS-CoV-2 of Vero cells in a 96-well plate in four replicates (viral dose, 100 TCID₅₀ per well) and incubated for 5 days at 37 °C in a 5% CO₂ atmosphere. In addition to the test samples, the following controls were included: cell control (uninfected cell culture), virus control (cells infected with a working dilution of virus), serum control (serum diluted 1:20), and dose control (fivefold dilutions of virus). The neutralization reaction result was recorded visually by microscopic examination of the cell monolayer on day 5. The neutralizing titer was defined as the reciprocal value of the last dilution at which no signs of CPE were detected in two or more wells.

Transmission electron microscopy

The samples were applied to glow-discharged TEM grids with carbon support film (TedPella Carbon Type B, 300 mesh). The grids were then negatively stained with 1% uranium acetate. TEM images were acquired using a JEOL JEM-2100 200-kV electron microscope (Japan) equipped with Gatan Orius SC200D camera (2k x 2k) (USA). Negatively stained samples were imaged at magnification yielding a 3.4-Å pixel size with −1.5 µm defocus applied.

Statistical processing of the data

Statistical data processing was performed using GraphPad Prism v.5.03 software. The correlation of virus titer and viral RNA concentration was evaluated using Spearman’s test at a 95% confidence interval. The significance of the difference was determined using Student’s t-test at a 95% confidence interval.

Work safety requirements

All work with SARS-CoV-2 was carried out under biosafety level 3 conditions.

Results

Virus accumulation and ultraviolet (UV) inactivation

The growth kinetics of SARS-CoV-2 strain Dubrovka, and viral RNA replication in Vero cells at different multiplicities of infection (MOI), 0.001 and 0.00001, were studied to determine the conditions for producing viral material with high titer. Plots of the accumulation of infectious virus and viral RNA in cells are shown in Figure 2.

With an MOI of 0.001, the virus titer reached maximum values by 36 hours postinfection (p.i.) and remained at 8.25-8.75 log₁₀ TCID₅₀/ml at 36-72 hours p.i., after which it decreased and reached 4.75 log₁₀ TCID₅₀/ml at 96 hours p.i. At an MOI of 0.00001, the virus titer reached a maximum of 9.0 log₁₀ TCID₅₀/ml at 48 hours p.i. It remained at this level until 84 hours p.i. and decreased to 6.5 log₁₀ TCID₅₀/ml at 96 hours p.i.

The pattern of viral RNA accumulation was generally consistent with the viral titer curve, except for the period after 84 hours p.i., when the viral titers started to decrease, whereas the RNA content remained at a maximum of approximately 11.0 log₁₀ RNA copies/ml until 96 hours p.i. The data obtained by titration correlated significantly with the results obtained by real-time RT-PCR between 12 and 84 hours p.i. (MOI 0.001, Spearman ρ = 1.000, P < 0.05, MOI 0.00001 Spearman ρ = 1.000, P < 0.05).

Statistical data processing was performed using GraphPad Prism v.5.03 software. The correlation of virus titer and viral RNA concentration was evaluated using Spearman's test at a 95% confidence interval. The significance of the difference was determined using Student's t-test at a 95% confidence interval.

Fig. 2 Growth kinetics of SARS-CoV-2 (Dubrovka strain) in Vero cells. (A) Virus titer. (B) Viral RNA concentration. Cells were inoculated at an MOI of 0.001 and 0.00001. Supernatant samples were collected every 12 hours to titrate the virus and determine the concentration of viral RNA.

Fig. 2 Growth kinetics of SARS-CoV-2 (Dubrovka strain) in Vero cells. (A) Virus titer. (B) Viral RNA concentration. Cells were inoculated at an MOI of 0.001 and 0.00001. Supernatant samples were collected every 12 hours to titrate the virus and determine the concentration of viral RNA.
For viral antigen production, Vero cells were infected with SARS-CoV-2 at an MOI of 0.00001, and the virus-containing supernatants were collected at 72 hours p.i., yielding viral material with a titer of 8.75 log10 TCID<sub>50</sub>/ml and a viral RNA concentration of 9.5 log10 copies/ml. The viral material was treated with UV light for different lengths of time, from 30 s to 12 min, and the degree of virus inactivation was assessed (Table 4).

Complete inactivation of the virus was observed upon irradiation for 4 minutes or more, which was confirmed in five independent experiments in which residual infectivity was tested by three blind passages in Vero cell culture. The survival rate of Vero cells inoculated with irradiated virus preparations increased with increasing irradiation time (Fig. 3).

It was found that UV exposure for 4, 8, and 12 minutes resulted in complete inactivation of the Altufjevo and Podolsk isolates of SARS-CoV-2, which belongs to Omicron and Delta variants of concern. In further work, viral material treated with UV light for 4 min was used to evaluate the antigenic and immunogenic properties of the virus.

### Structural characterization of UV-inactivated SARS-CoV-2

In the UV-inactivated SARS-CoV-2 (Dubrovka strain) preparation, TEM revealed virus-like particles with morphodiagnostic features of a coronavirus. The virions had a round shape with characteristic 12- to 15-nm spikes on the shell, and the virion diameter was 90-110 nm (Fig. 4). The shape and size of these virus-like particles are in good agreement with the previously obtained microphotographs of β-propiolactone-inactivated particles of SARS-CoV-2 [5].

| Exposure time, min | 0.0 | 0.5 | 1 | 2 | 4 | 8 | 12 |
|-------------------|-----|-----|---|---|---|---|----|
| Virus titer (log<sub>10</sub> TCID<sub>50</sub>/ml) | 8.75 ± 0.27 | 6.75 ± 0.18 | 4.0 ± 0.40 | 2.0 ± 0.21 | n/d | n/d | n/d |
| Residual infectivity (%) | 100 | 1.0 | 1.8 × 10<sup>-2</sup> | 1.8 × 10<sup>-5</sup> | 0.0 | 0.0 | 0.0 |
| Degree of inactivation (%) | 0.0 | 99.00 | 99.98 | >99.99 | 100 | 100 | 100 |

n/d - not detected

Fig. 3 Survival of Vero cells on day 5 after inoculation with a UV-inactivated SARS-CoV-2 preparation. The virus preparation was irradiated for the indicated times at a UV-C intensity of 290 μW/cm<sup>2</sup>. Inactivation of the virus was confirmed by blind passage in Vero cells. Vero cell survival was measured using an MTT test.

Fig. 4 Electron micrograph of negatively stained UV-inactivated SARS-CoV-2 at 40 000× magnification. The arrow shows characteristic spikes (S-protein) on the surface of the coronavirus. Panels A and B represent different fields of view for the same preparation of the Dubrovka strain.
Immunogenic properties of UV-inactivated SARS-CoV-2

The antigenic properties of the virus after UV treatment were assessed using a rapid immunochromatographic (IC) test for the presence of the SARS-CoV-2 antigen and by ELISA. In the UV-inactivated SARS-CoV-2 (Dubrovka strain) preparation, the viral antigen was detected up to a dilution of 1:15,625 by IC, whereas by ELISA it was detected up to a dilution of 1:78,125 (Fig. 5, Table 5).

The Dubrovka strain was chosen for the study of immunogenicity of UV-inactivated SARS-CoV-2, since it had the highest growth rate and titer (Table 1). It was observed that mice immunized with Freund’s adjuvant had lost significant weight. On day 8 after the second immunization, the difference in weight compared to the control group was 12% ($p < 0.05$), while the mice in the other groups did not differ in weight from those in the control group.

After the first immunization, the blood of animals immunized with UV-inactivated SARS-CoV-2 with Freund’s adjuvant and aluminum hydroxide showed detectable amounts of antibodies to SARS-CoV-2 by ELISA. After the second immunization, antibodies to the virus were detected in the blood of immunized animals of all groups, with the highest titers of antibodies achieved when Freund’s adjuvant (13120 ± 8497) or aluminum hydroxide (1320 ± 1163) was included in the preparation (Fig. 6A).

The main criterion for evaluating immunogenic properties was the ability of UV-inactivated SARS-CoV-2 to induce neutralizing antibody (NAb) production in animals. After the first immunization, NAbs were detected in the blood of animals immunized with UV-inactivated SARS-CoV-2. After the second immunization, NAb titers increased by an average of 20- to 40-fold. NAb titers were significantly higher in mice immunized with a preparation containing Freund’s adjuvant (448 ± 520) than in mice immunized with preparations containing aluminum hydroxide (96 ± 128) or without adjuvant (62 ± 261), $p < 0.01$ (Fig. 6B).

In the group of mice injected with live virus, 2 weeks after immunization, antibodies to SARS-CoV-2 were detected in both ELISA (2760 ± 561) and the neutralization assay (308 ± 544), and at a higher titer than when the
corresponding dose of UV-inactivated SARS-CoV-2 with different adjuvants was injected \((p < 0.01)\) (Fig. 6).

In the control group \((K-)\) animals, no antibodies to the virus were detected by ELISA or in the neutralization assay (Fig. 6).

**Discussion**

Inactivated virus preparations have traditionally been used in the production of vaccines [24]. Inactivated vaccines are safe and effective for preventing influenza, polio, hepatitis A, tick-borne encephalitis, Japanese encephalitis [25–29], and COVID-19 [5, 16–20]. In most cases, inactivated vaccine production technology involves chemical inactivation of the virus. However, there are examples of the use of ultraviolet radiation for the production of promising vaccine preparations against SARS-CoV, herpes simplex virus, porcine epidemic diarrhea coronavirus, measles virus, rabies virus, influenza virus, and monkey immunodeficiency virus [30–36]. A high efficiency of a UV-inactivated preparation of rabies virus for emergency prevention of rabies has been reported [34]. A UV-inactivated preparation of SARS-CoV in the work of Iwata-Yoshikawa et al. [37] protected immunized mice against challenge with a wild strain of the virus. Ultraviolet light disinfection has been used successfully in medical facilities and laboratories dealing with SARS-CoV-2 [38] and other pathogens. However, the use of UV inactivation of SARS-CoV-2 for vaccine production has not been described in the scientific literature.

Ultraviolet radiation is divided into three wavelength ranges: UV-A (320-400 nm), UV-B (280-320 nm), and UV-C (200-280 nm). UV-C is the most powerful ultraviolet radiation of the three, and is used for disinfection [38–40]. Therefore, in our work, we treated viral material with UV-C at 253.7 nm. UV radiation causes photochemical damage to viral nucleic acids by inducing dimerization of adjacent pyrimidine nucleotides, which disrupts the transcription and replication of the viral genome [40]. Since RNA-containing viruses usually have ineffective mechanisms of genome repair, they can be easily inactivated by UV radiation [38–40]. It is important to mention that ultraviolet light effectively inactivates viruses while preserving the integrity of epitopes, which allows the use of preparations of UV-inactivated viruses in the production of diagnostic test systems and vaccines [33, 34, 37, 39, 40]. Since UV irradiation causes non-specific damage to viral RNA, we believe that the results obtained on three antigenically different strains of SARS-CoV-2 (Dubrovka, Altufjevo, and Podolsk) are applicable to other SARS-CoV-2 strains.

Widely used chemical methods of virus inactivation involve the use of highly toxic substances such as \(\beta\)-propiolactone, classified as a potent carcinogen to humans (group 2B carcinogen), and formaldehyde, classified as carcinogenic to humans (group 1 carcinogen) by the International Agency for Research on Cancer [41]. For this reason, physical methods of virus inactivation such as UV
irradiation are preferred in vaccine production technology, as they do not involve the treatment of the virus with harmful substances and therefore do not require the introduction of additional steps to purify the viral antigen from toxic compounds.

In this study, ELISA and IC methods revealed the ability of UV-inactivated SARS-CoV-2 to bind to antibodies in convalescent sera at high dilutions of viral antigen up to 1:78,125 (Table 4), indicating that the antigenic determinants were preserved. When injected subcutaneously into mice, UV-inactivated SARS-CoV-2, both in its free form and complexed with adjuvants, induced the production of antibodies to structural proteins of the virus, including antibodies directed to neutralizing epitopes of the S protein. The ability of UV-inactivated SARS-CoV-2 to induce the production of neutralizing antibodies as the main indicator of specific protective activity for whole-virion-inactivated vaccines was highlighted in this work. Furthermore, SARS-CoV-2, inactivated at the minimum dose required for inactivation by UV irradiation, was used for immunization to minimize damage to the antigenic determinants of viral antigens. TEM revealed coronavirus virions with highly conserved spikes consisting of the S protein in the UV-inactivated SARS-CoV-2 preparation, which is consistent with the observed immunogenicity of the preparation.

The results showed that the immunogenicity of UV-inactivated SARS-CoV-2 can be enhanced by the use of adjuvants, with Freund’s adjuvant being the most effective. However, the immunogenicity of live SARS-CoV-2 in mice was higher than that of UV-inactivated SARS-CoV-2 variants, even when injected in the presence of adjuvants (Fig. 6). Although SARS-CoV-2 is not pathogenic to mice, it readily adapts to reproduce in mouse lungs and is capable of inducing a distinct humoral immune response in animals [42–45]. Therefore, it seems reasonable that the infection of mice with an infectious virus induces a stronger humoral immune response than the administration of a corresponding dose of UV-inactivated SARS-CoV-2.

**Conclusion**

Treatment of SARS-CoV-2 with UV light completely inactivates its infectivity while preserving its morphology, antigenic properties, and ability to induce the production of virus-neutralizing antibodies in mice after immunization. Thus, the inactivation of SARS-CoV-2 by UV makes it possible to obtain viral material similar in its antigenic and immunogenic properties to the native antigen, which can be used both for diagnostic purposes (ELISA, immunoblotting, IC assay) and for the development of an inactivated vaccine against COVID-19.

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**Author contributions** All authors contributed to the study’s conception and design. Material preparation, data collection, and analysis were performed by Gracheva A.V., Faizuloev E.B., Korchevaya E.R., Smirnova D.I., and Samoilikov, R.V. Electron microscopy was performed by Sokolova O.S, Glukhov G.S, Moiseenko A.V. and Zubarev I.V. The first draft of the manuscript was written by Gracheva A.V., Faizuloev E.B., and Ammour Yu. I., and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animals, including the Guide for the Care and Use of Laboratory Animals [46], were followed. This study was approved by the Medical Ethics Review Committee of the I. I. Mechnikov Research Institute of Vaccines and Sera (Ethics Committee Decision No 2 dated May 24, 2021).

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