ISSN: 2320-2831

International Journal of Pharmacy and Analytical Research (IJPAR)

IJPAR |Vol.14 | Issue 3 | Jul - Sept -2025

www.ijpar.com

DOI : https://doi.org/10.61096/ijpar.v14.iss3.2025.820-825

Research

A Sars Coronavirus Vaccine, A Double-Inactivated Whole Virus Candidate, Produces Protective and Negative Antibody Responses

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Published on: 22 Sep 2025

Published by:
Futuristic Publications

Abstract

Using fermenter cultures of serum protein-free Vero cells, a double-inactivated candidate whole virus vaccine against the severe acute respiratory syndrome associated coronavirus (SARS-CoV) was developed and produced on a large scale. To provide a very high safety margin with regard to residual infectivity, a two-step inactivation process that involves successive formaldehyde and UV inactivation was used. This double-inactivated vaccine's immunogenicity has been tested using a mouse model. Mice that given two doses of the proposed SARS-CoV vaccine showed high levels of neutralizing antibodies and antibody titres against the SARS-CoV spike protein. Only a small adverse effect on the vaccine's immunogenicity was caused by the addition of the adjuvant Al (OH)3. In addition, immunization induced cell-mediated immunity as demonstrated by interleukin-4 and interferon stimulation. Additionally, the vaccination gives protective immunity, wherein shown by stopping SARS-CoV multiplication in the mice's respiratory tracts following an intranasal SARS-CoV challenge. Antibody titres against the SARS-CoV S protein and neutralizing antibody titres were linked to mice's protection.

Keywords: SARS-CoV; whole virus vaccine; double-inactivation; formaldehyde; UV inactivation; Vero cells; neutralizing antibodies.

1. INTRODUCTION

A novel disease known as severe acute respiratory syndrome developed in China in late 2002 and quickly swept across the world. With almost 8000 illnesses and over 800 casualties, this global epidemic sparked widespread worries that this new agent posed a major threat to public health around the globe. Due to their capacity to elicit powerful humoral and cellular immune responses, live attenuated viruses are often regarded as the most

effective viral vaccines [5]. However, the debut of such candidate vaccinations into clinical trials may be delayed due to the requirement for comprehensive safety assessment. Antibodies against the spike (S) glycoprotein have been shown in numerous investigations to interfere with SARS-CoV infectivity [6–9]. According to reports, the S protein mediates viral entrance into cells via binding to the human angiotensin c converting enzyme-2 (hACE-2) protein [10]. According to reports, human monoclonal antibodies that target the S protein can fully stop the development of SARS Co-V-induced lung pathology and decrease the virus's ability to replicate in infected ferrets' lungs [11]. Furthermore, several potentials

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2.1 VIRUS STRAIN

The CDC (Atlanta, USA) provided the virus strain SARS-CoV CDC#200301157, commonly known as strain Utah; GenBank accession number AY714217. It was isolated from a SARS patient's sputum in the United States (specimen #809940) and passed through two rounds of passage on GMP-grade Vero cells.

2.2. WESTERN BOLT AND ELECTROPILIC ANALYSIS.

Purified SARS-CoV vaccine denatured samples (about 1 g protein/lane) were separated under reducing conditions on a 10% Tris–HCl sodium dodecyl Sul fate–polyacrylamide gel before being blotted onto a PVDF membrane. Following the manufacturer's instructions, protein staining was carried out using the Auro Dye TM forte staining kit (Amersham Bioscience/GE Healthcare, UK). Rabbit antibodies directed against the SARS-CoV nucleocapsid (N) protein (IMG-548) or I'm gene (San Diego, CA, USA) provided the S protein (IMG-557), which was utilized for Western blot analysis. Membranes were cleaned and incubated for one hour at room temperature with goat anti-rabbit IgG (Accurate Chemical, Westbury, USA) labelled with horse radish peroxidase (HRP) following blocking and application of the primary antibody overnight at 2–8◦C (working dilution: 1:1000). Blots were created using 3,3-diaminobenzidine tetrahydrochloride and H2O2 after the last washing.

23 IMMUNICATION OF MICE

2.3.1 IMMUNICATION OF CDI MICE

Six to eight-week-old female CD1mice were acquired from e Charles River Laboratorie subsidiary located in Sulz fled, Germany.

Following pre-immune serum sampling, mice received six doses of the candidate vaccine, ranging from 1 g to 0.3 ng of total protein (Bradford's technique [16]). A non-adjuvanted preparation and material adjuvanted with 0.05 and 0.2% aluminium hydroxide (alum) were evaluated at each antigen concentration in order to assess the impact of adjuvant Tionon immunogenicity.

Ten mice were subcutaneously (sic) inoculated with 0.5 ml of each vaccination formulation. The same volume of buffer (Tris-buffered saline) was given to the control groups with or without 0.2% aluminium hydroxide. Each animal's sera were extracted fourteen days after the initial vaccination, and a booster shot was administered using the same dosage and immunization as the primary immunization. Four weeks following primary immunization, sera were extracted from each animal (those receiving the vaccine containing 0.2% aluminium hydroxide were also bled twelve weeks following primary immunization).

2.3.2 IMMUNICATION OF BALB MICE

We also acquired Bulb/C mice (6–7 weeks old) from the Charles River Laboratories subsidiary. Groups of 15 Bulb/C mice were vaccinated such with 1 g of vaccine adjuvanted with 0.2% alum for cytokine studies. 14 and 28 days after the initial vaccination, booster shots containing the same formulation were administered. Five mice per group were slaughtered by cervical dislocation 21 and 35 days following the initial vaccination. By pushing chopped tissue through 200 mesh stainless steel sieves, a single cell suspension is created. Leukocytes were suspended in full cell culture medium after red blood cells were depleted by incubating them for five minutes at room temperature with lysis buffer that contained 0.15M NH4Cl, 10mM KHCO3, and 0.1M Na2-EDTA, pH 7.4.

2.4 CHALLENGE OF CDI MICE

A subset of the CD1 mice that had been immunized, specifically four mice each from the groups that received vaccine doses of 0.2 g–0.3 ng and the control groups (refer to Section 2.3.1), were challenged either 13 weeks (0.2% aluminium hydroxide) or 5 weeks (groups that received the non-adjuvanted or 0.05% aluminium hydroxide containing preparation) following primary immunization.

A blood sample was obtained in order to determine the neutralizing antibody titres prior to challenge. Mice were given an intranasal inoculation of 105 TCID50 SARS-CoV in a volume of 20 l (10 l per nostril) after being anesthetized with isoflurane for the challenge. For the homologous challenge, non-cloned SARS-CoV CDC#200301157, which has been multiplied five times on serum protein-free Vero SF cells, was utilized.

2.5 ENZYME LINKED IMMUNOSORBANT ASSAY [ELISA]

An indirect ELISA was utilized to ascertain the IgG titre against the S protein of SARS-CoV. In short, 100ng of Baculovirus (BV)-expressed, full-length, His-tagged S protein (Protein Sciences, Meriden, CT, USA) in 50mM carbonate buffer, pH 9.6 per well, or uncoated, respectively, were applied to 96-well microtiter plates overnight at 2–8◦C. Serial fourfold dilutions of sera (beginning with a 1:100 dilution) were applied for one hour at room temperature after washing and blocking for one hour with 3% non-fat dry milk. This was followed by three washes with phosphate-buffered saline (PBS) containing 0.1% Tween 20. Goat anti-mouse IgG labelled with HRP (Accurate Chemical) was used to identify bound antibodies.

2.6 NEUTRALIZATION ON THEIR DETERRMINATION

Serum samples were serially diluted with cell culture media in twofold steps (typically commencing at a dilution of 1:20 or 1:40). After mixing the serum dilutions 1:1 with a viral stock suspension that had been adjusted to 103.5 TCID50/ml, they were allowed to sit at room temperature for one hour before being transferred (eight replicates per dilution) to a 96-well tissue culture plate that had been seeded with Vero cells. Before the cultures were examined under a light microscope for the presence of a cytopathic effect (CPE) brought on by SARS-CoV, such as cell rounding and detachment, the plates were incubated for five days at 37◦CinaCO2-incubator. The neutralising titre was calculated by the number of virus negative wells and the serum dilution according to the method of Spearman

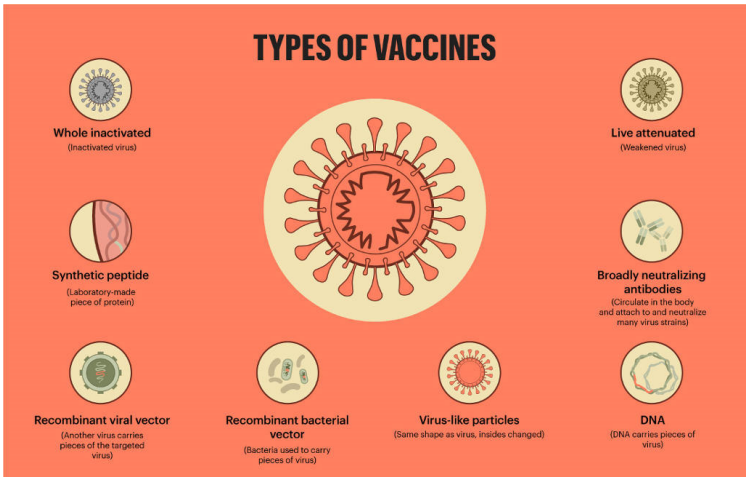
2.7 THE ELISPOT TEST T

Mouse IFN-nd IL-4 ELISPOT kits (Matich AB, Nacka, Sweden) were used to analyse the frequency of cells that secrete interleukin-4 (IL-4) or interferon (IFN-), in accordance with the manufacturer's instructions. Freshly extracted spleen cells from Blab/C mice were serially diluted and put to antibody-coated 96-well plates in wells ranging in size from 5×104 to 2×105 cells. SARS candidate vaccine and recombinant BV-expressed S protein were introduced at concentrations of 0.1–1 g/ml for stimulation As negative and positive controls, respectively, wells containing no antigen or 1 g/ml of pokeweed mitogen (Sigma, St. Louis, USA) were employed. Following an overnight incubation period at 37°C and 5% CO2, the cells were disposed of and the plate were cleaned with PBS. Following streptavidin-alkaline phosphatase and development with BCIP/NBT substrate solution, a biotinylated interferon-or IL-4 specific antibody was used to detect interferon-or IL-4, respectively. The number of spots was counted using an auto mated ELISPOT reader (AID, Strassberg, Germany). Spot forming cells (SFC) per 106 spleen cells were the result of subtracting the number of spots seen in wells with no antigen from the number of spots seen in wells with a particular antigen.

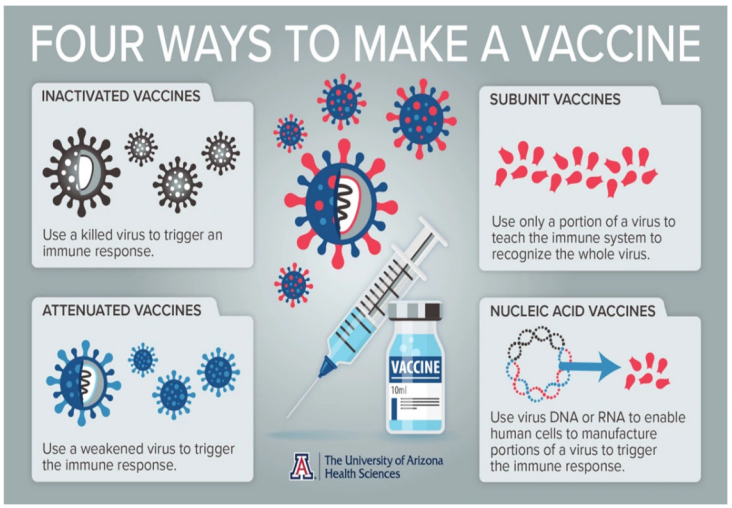
2.8 TISSUE CULTURE INFUCTIONS DOSE 50%

A TCID50 assay was used to quantify the infectious virus titre of SARS-CoV-containing samples. To put it briefly, 96-well microtiter plates seeded with Vero cells were inoculated with repeated 10-fold dilutions of virus-containing samples. Following five to seven days of incubation at 37°CinaCO2, the plates were examined under a light microscope to check for the presence of a CPE. Using an internal calculation software program, the TCID50 was determined using the Poisson formula based on the number of virus-positive wells per dilution. This application uses the one-hit model to estimate the titres' TCID50 and 95% confidence limit

TYPE OF VACCINES



WAYS TO MAKE VACCINES



3. RESULTS

3.1 DEVALOPEMENT OF FORMENTATION, INACTIVATION AN PURIFICATION PROCESS

Using large-scale serum protein-free Vero cell fermenter cultures, we previously reported on the production of a whole viral influenza vaccine that is formalin inactivated [15]. In order to create ideal conditions for the growth, inactivation, and purification of the inactivated virus, a candidate SARS-CoV vaccine was developed based on adaptation of this well-established technology. Vero cells were thought to be the best cell matrix for quick vaccine production because the SARS-CoV had been shown to grow well on them [2, 3]. Five consecutive plaque cloning's from a human isolate produced a main viral seed. This primary was further amplified to generate.

A seed virus bank, a working virus bank and a pro duction virus bank. 100l fermenter volume was then used to infect serum protein-free cultures with the production virus. After two to three days of incubation, the virus-containing supernatant was extracted and rendered inactive by 48 hours of treatment with 0.05% formalin, followed by UV inactivation at a dosage of 20 mg./cm2. High viral titres (approximately 108 TCID50/ml) were generated in the supernatant following virus infection at a multiplicity of infection (mo.) of 0.001, and both the formalin and UV inactivation steps were independently capable of inactivating this virus with a high margin of safety (manuscript in preparation).

3.2 ANTIBODY RESPONS THAT IS SPECIFIC AND NEUTRALAIZING

The proposed vaccine's immunogenicity was first examined in dose-finding and adjuvant tests conducted on CD1 mice. This was accomplished by immunizing groups of ten mice twice with progressively lower doses of the purified adjuvanted or non-adjuvanted vaccine, ranging from 1 g to 0.3 ng. A non-adjuvanted preparation and material adjuvanted with 0.05 and 0.2% aluminium hydroxide were tested at each antigen concentration in order to assess the impact of adjuvant on the vaccine's immunogenicity.

The titres of the neutralizing and S-specific antibodies Two weeks following the first vaccination, measurements were made of roughly 1:5000 for the adjuvanted formulations and 1:1000 for the non-adjuvanted formulations (data not shown). At this moment, neutralizing antibody titres were notassessed. After receiving the booster shot, ELISA titres significantly rose, and the 1 g dosage adjuvanted with 0.2% aluminium hydroxide allowed for GMTs of up to 1:400,000.

3.3 DETERMINING E.D 50 WITH AN WITHOUT AN AJUVANT

The effect of adjuvant on S-specific antibody responses was also ascertained by computing the effective dose 50 (E.D.50), which is the smallest quantity of antigen necessary for 50% of mice to undergo seroconversion following two vaccinations.

Following a booster vaccination, the non-adjuvanted and the vaccine containing 0.2% alum had a fairly similar antibody response, as shown by the data in Table 1A; the adjuvanted (0.2% alum) and non-adjuvanted vaccines had E.D.50 values of 0.8ng and 1.1ng, respectively. The vaccination was marginally more immunogenic after being adjuvanted with 0.05% alum. Even at the lowest dose administered, 0.3 ng, 70% of the mice generated specific antibodies against the S protein.

3.4 RESEARCH ON PROTECTION

The findings reported in indicates again that the non-adjuvanted vaccine and vaccination preparations adjuvanted with 0.05% aluminium hydroxide were highly successful in eliciting specific anti-S antibodies and neutralising antibody is. In 3 out of 4 or 4 out of 4 immunized mice, respectively, a delayed antigen dosage with or without an adjuvant was successful in generating neutralizing antibodies.

Challenge with 105 TCID50 of live virus resulted in virus replication in the lung of 100% of mock immunised mice. However, vaccinated mice showed a good level of protection (3 of 4 and 4 of 4 animals protected) were immunized with candidate vaccine antigen dosages as low as 8ng. Reduced resulted in partial protection with ≥50% of immunised mice being protected following immunisation with 1.6ng of vaccine antigen. Similar data were obtained for vaccine preparations adjuvanted with 0.2% aluminium hydroxide protective dose (P.D.50) was calculated for all three vaccine formulations and were shown to be approximately equal with P.D.50's of 1.4, 0.7 and 1.0ng being calculated for formulations without adjust vent, with0.05%alumand0.2%alum, respectively.

3.5 CORRELATES OF PROTECTION IN SEROLOGY

According to the results, there was a definite relationship between a certain IgG titre and S protein. neutralizing antibody titration and defines against live virus intra-nasal challenge. This information was verified by summarizing all of the mouse model's protection data. The data collected for 168 mice used in various immunization studies during this development is summarized in Table 4. Table 4A shows that mice challenged with 105 TCID50 of live virus were 100% protected by a specific anti-Sigg titre of ≥25,600. Additionally, it was shown that 100% protection against challenge was achieved with a specific neutralizing titre of 114 (Table 4B). No virus replication was seen in mice with antibody titres higher than this. On the other hand, 105 TCID50 of the live virus intranasal challenge infected 89% of the control mice.

4. CONVERSATION

The thorough immunological characterization of a prospective SARS coronavirus vaccine is described in this research. Several distinct approaches have been documented in the past for the creation of experimental and candidate human SARS vaccines. These consist of DNA vaccines [14], attenuated viral vectors producing SARS CoV proteins [7,8,12,13], and inactivated whole virus vaccines [19–20]. We have favored the entire virus vaccine development approach for several reasons, including development speed. The virus develops to extremely high levels in Verocells, a cell line that is recognized by the majority of regulatory bodies.

CONCLUSION

The study demonstrates that a double-inactivated whole virus SARS-CoV vaccine, developed using Vero cell cultures and inactivated through formaldehyde and UV treatment, is capable of eliciting strong immune responses in mice. The vaccine induced high titres of neutralizing antibodies against the spike protein, as well as significant cell-mediated immunity (IL-4 and IFN responses). Even at low antigen doses, the vaccine provided protective immunity by preventing SARS-CoV replication in the respiratory tract following intranasal challenge. The addition of aluminium hydroxide showed only a minor effect on immunogenicity. Overall, the candidate vaccine proved safe, highly immunogenic, and protective, supporting its potential for further development as a SARS-CoV vaccine.

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