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ORIGINAL ARTICLE

Antigenicity and immunogenicity of SARS-CoV-2 surface glycoprotein fragment in CHO cells

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ABSTRACT

Background and Objectives: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) glycoprotein that projects from the virus surface is highly immunogenic. It is considered to be the target of many neutralizing antibodies as well as a target in vaccine design efforts. Evaluation the immunogenicity of a recombinant fragment of the spike protein (rfsp) that is comprised of Receptor Binding Domain (RBD), S1/S2 cleavage site, and fusion peptide (FP) as immunogenic proteins of SARS-COV-2, in BALB/c mice and evaluation of the efficacy of epitopes rfsp as a multi-subunit chimeric vaccine.

Materials and Methods: The present study made use of CHO-K1 (Chinese hamster ovary K1) cells to create a cell line for constant expression rfsp. The rfsp was purified with Ni-NTA chromatography and confirmed by Western blotting. The immunogenicity and neutralizing antibody efficacy of rfsp were evaluated in BALB/c mice. ELISA was employed to test rfsp via sera of COVID-19 convalescent patients infected with SARS-CoV-2 alpha and delta variants.

Results: Our results showed significant differences in antibody titers in immunized mice compared to the control groups and neutralizing antibodies were positive, sera from mice immunized are capable of bound SARS-CoV-2 virus, chimer peptide is capable bound antibodies patients infected with SARS-CoV-2 and patients infected with delta variant SARS-CoV-2.

Conclusion: Overall, these results indicate that rfsp protein would be a novel potential antigen candidate for the development of a subunit SARS CoV-2 vaccine and rfsp has the potential to be a useful option for the development of the assays for serodiagnosis of SARS-CoV-2 infection.

Keywords: Spike; Vaccine; CHO-K1; SARS-CoV-2; Delta variant

INTRODUCTION

The appearance of COVID-19 was caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Wuhan, China, and quickly spread throughout the world (1). The pandemic of COVID-19 has brought about a worldwide health crisis and enormous economic failure (2). Therefore,

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the production of an efficient vaccine is essential to control the SARS-CoV-2 pandemic (1). The first defense line against the SARS-CoV-2 infection that caused the coronavirus disease 2019 (COVID-19) pandemic is the immune response (3). Projecting from the surface of the virus, the spike glycoprotein is highly immunogenic. It is the target of many neutralizing antibodies (4) and a target in vaccine design efforts (5). It consists of two protein subunits (S1 & S2) (6, 7). S1-protein is comprised of RBD, which identifies angiotensin-converting enzyme 2(ACE2) (8). The S2-protein has the FP (9). The viral spike protein bears an S1/S2 cleavage site. The spike protein cleaved by the cellular furin protease at the S1/S2 site is critical for entry into human lung cells and S-protein-mediated cell-cell fusion (10). Both the spike protein and the ACE2 receptor are heavily glycosylated. They are, for instance, glycosylated at sites near their binding interface and the role of glycosylation in binding has been previously proved (11). Variants have been recognized with the use of sequencing and the spike (S) gene status. Recently, the worldwide increase in Spike variants has raised concerns about the continuing efficacy of the vaccine (12). Some mutations are located at the interaction surface between the human angiotensin ACE2 and RBD. For instance, the B.1.617.2 (delta) variant (13) has affected the respiratory organoids and other cells by increasing the entry efficiency associated with the wild-type Wuhan D614G spike (14). The S protein contains 22 potential N-glycosylation sites (15). Finding out the glycosylation mechanism of recombinant viral spikes can disclose fundamental features of viral biology and direct vaccine design strategies (6). Viral glycosylation plays a wide range of roles in viral pathobiology, such as mediating protein folding and forming viral tropism (16). The mammalian cell system, has a better chance of expression folded and soluble proteins with proper glycosylation, allowing the expressed protein to keep its bioactivity and native structure (17). Moreover, there are other advantages of using a eukaryotic host including advanced production of recombinant proteins with post-translational modifications, and the potential to be cultured at production-scale volumes (18). In this regard, Chinese hamster ovary (CHO) cells are often used for the production of recombinant proteins in a mammalian system (19). DNA and RNA delivery into cultured cells is an important transgenic technique in molecular biology (20). Currently, three basic techniques have been applied to perform this mission including calcium phosphate, lipid-mediated, and electroporation (21). The current study made use of electroporation to transfect recombinant DNA into CHO-k1 cells. The rfsp was expressed in CHO-k1 cells and purified by Ni-NTA chromatography. The purified rfsp was characterized by Western blot and evaluated by the Indirect Enzyme-linked Immunosorbent Assay (ELISA) using sera of SARS-CoV-2 patients. The immunogenicity of rfsp and its ability to neutralize antibodies were evaluated in immunized BALB/c mice.

MATERIALS AND METHODS

Human and animal rights. All protocols of the study were approved by the institutional animal ethics committee of Baqiyatallah University of Medical Sciences (IR.IAU.SRB.REC.1400.075) which follows the NIH guidelines for care and use of animals.

Construct design. The gene sequences and spike protein S [NCBI Reference Sequence: -YP_009724390.1] of SARS-CoV-2 were obtained from the NCBI using in silico analyses. The chimeric construct included RBD that was attached to domain S1/S2 cleavage and linked to the FP protein. The rfsp sequence was synthesized as a clone into the pUC57 vector by ShineGene Molecular Biotech.

Primer design. Oligo 7 tool was used to design two sets of primers (Table 1), which were synthesized by Cinaclon, Iran.

Preparation of linearized pcDNA3-rfsp vector. The full-length rfsp (1.2 kb) cDNA was cleaved with *Hind*III and *Eco*RI from the puc57 vector, then subcloned into the pcDNA3 (+) vector containing the CMV (cytomegalovirus) promoter. The resultant pcDNA3-rfsp plasmid was transfected into competent *Escherichia coli* TOP10. Individual colonies were transferred into ampicillin (50 mg/ml) LB medium and incubated overnight at 37°C with vigorous shaking (250 rpm on a rotary shaker) until the bacteria reached the late log phase. Recombinant plasmid pcDNA3-rfsp was extracted using alkaline lysis with SDS, linearized with SspI, purified with primePrep Gel purification Kit (GeNet Bio), and recovered with deionized distilled H_2O or 1 × TE buffer (pH 7.6).

Table 1. The sequences of the primers used in this study.

Sequence name	Target gene	Primer sequence 5´→3´	PCR product (bp)
rfSP	spike	spike forward: ATATATGAATTCGCCACCATGGTGAGGGT spike reverse: ATAGATAAGCTTCAGGCCGTTGAACTTCTGG	1200
pcDNA 3.1	pcDNA 3.1	pcDNA3.1 forward: TAATACGACTCACTATAGGG pcDNA3.1 reverse: GCAATTTCCTCATTTTATTAG	300

Cell culture. The cells which were considered for transfection were cultured at 37°C in a humidified incubator with an atmosphere of 5% CO_2, in an RPMI high glucose medium (pH 7.4, Gibco) supplemented with 10% inactivated fetal bovine serum (10% FBS, Invitrogen), 100 U/ml penicillin, and 100 mg/ml streptomycin (1% Pen-Strep, Invitrogen). CHO-K1 (NCBI Code: C644, Chinese hamster ovary K1) cell lines from the Pasteur Institute of Iran were used in the study. With a seeding density of 2.5×10^5 cells/ml, the cells were passaged every 3-4 days. They were transfected with expression plasmids at 50% confluence, then sub-cultivated at 80%-90% of cell confluence.

Transfection and preparation of stable clones. Before conducting electroporation, CHO-K1 cells were pelleted at 250 × g for 10 min, suspended at 2×10^8 cells/mL in Gene Pulser electroporation buffer (Bio-Rad, Hercules, CA), and mixed with recombinant Plasmid DNAs pcDNA3-rfsp (1-2 µg DNA per 1×10^6 cells). Next, cells were electroporated using the "CHO" protocol provided with the Gene Pulser Xcell Electroporation Systems (Bio-Rad) voltage was 160 V and pulse duration was 15 ms, immediately transferred into flasks, and incubated for 24 hours at 37°C in a 5% CO_2 incubator. Twenty-four hours after transfection, the cells were washed and a fresh medium was added to the cells. Forty-eight hours after transfection, the cells were split into fresh medium containing 600 µg/mL geneticin and were fed with selective medium every 3-4 days, upon which the individual clones were monitored for rfsp production during the predetermined 21 days. PCR reaction was used to analyze the colonies.

PCR reaction. To ensure the rfsp gene presence in the pcDNA plasmid PCR reaction was done using pcDNA 3.1 primers according to the manufacturer's instruction were used at a final concentration of 200 nmol/L: 5′- TAATACGACTCACTATAGGG -3′ (FW) and 5′- GCAATTTCCTCATTTTATTAG -3′ (REV) and pcdna3-rfsp vector as the PCR template (annealing temperature 56°C) to confirm the rfsp gene presence in the CHO cell genome. The transfected CHO cell genome was extracted by using Monarch® Genomic DNA Purification Kit (NEB #T3010) according to the manufacturer's protocol. After extraction of the transfected CHO cell genome, PCR reaction was performed using specific primers rfsp according to the manufacturer's instruction were used at a final concentration of 200 nmol/L: 5′- ATATATGAATTCGCCACCATGGTGAGGGT-3′ (FW) and 5′- ATAGATAAGCTTCAGGCCGTTGAACTTCTGG -3′ (REV) and the transfected CHO genome at different dilutions of 50, 1: 100, 1: 200, 1: 500was used as the PCR template (annealing temperature 65°). we used the HotStarTaq DNA Polymerase (Qiagen). The PCR was set up according to the manufacturer's instructions and run on a standard thermocycler. PCR products were visualized on a 1% w/v agarose gel stained with ethidium bromide.

Preparation of recombinant rfsp protein. CHO-K1 cells expressing rfsp were treated when they reached 80% confluence in T75 flasks. After washing the cells with 5 mL of PBS, we trypsinized the cells with 1.5 mL of trypsin to avoid using a cell scraper. They were then incubated for 5 min. Next, 3.5 mL of complete culture medium (RPMI high glucose medium (pH 7.4, Gibco) supplemented with 10% inactivated fetal bovine serum (10% FBS, Invitrogen), 100 U/ml penicillin, and 100 mg/ml streptomycin (1% Pen-Strep, Invitrogen) was added to the trypsinized cells. The centrifugation was done for 5 min at 1200 rpm at 4°C. After removing the supernatant, the washing and centrifugation were repeated. Once the PBS supernatant was removed, the cells overexpressing rfsp were suspended in a 300 µL RIPA buffer. The cells are kept on ice to delay protein degradation. A

protease inhibitor cocktail (1%) was also added to the mixture (protease inhibitor cocktail in DMSO, Santa Cruz Biotechnology). Then, sonication (6-10 s with 10-second pauses at 100-200 W) was applied to ensure complete cell lysis. The lysate was prepared for collection, by separating the debris from the sup using centrifugation. The centrifugation was done for 10 min at 15000 rpm at 4°C. 15,000 rpm at 4°C for 10 min. The supernatant was purified according to the Immobilized Metal Affinity Chromatography (IMAC) column using Ni-NTA agarose (Qiagen). The concentration of protein was indicated by the Bradford technique with BSA (bovine serum albumin) as a standard. Western blotting was applied to verify rfsp expression.

Western blotting. The cell lysate containing the recombinant rfsp was separated by 12% SDS-PAGE and electro-transferred onto PVDF membrane (Roche). The membrane was blocked using 5% non-fat skim milk in TBS buffer (50 mM Tris-Cl, 150 mM NaCl, pH: 7.5) with 0.05% Tween 20 (37°C, 2 h). Then, incubation was done with HRP-conjugated mouse anti-poly His-tag antibody (1:2000 Roche). was used to incubate the membrane. For signal development, Stain the membrane with 0.05% 3, 3'-Diaminobenzidine tablet (DAB Reagents; Sigma) 0.015% H_2O_2 in 0.01 M PBS, PH 7. 2. Bands will appear after 2 minutes.

Mice immunization. Ten 6-8 week old BALB/c mice (20-25 gr) purchased from the Razi Institute, Tehran, Iran, were divided into two groups: control and test groups. The animals were immunized three times. The first vaccination was performed subcutaneously with 15 µg of refolded rfsp mixed with the same volume of complete Freund's adjuvant. The second and third doses were injected as boosters of 15 µg of protein with incomplete Freund's adjuvant subcutaneously (at 15-day intervals). The control group received only PBS and adjuvant. Blood samples were collected on days 29 and 44 from the eye corners of mice with a capillary; the sera separated from blood samples were stored at -20°C until use.

Determination of serum IgG titer. ELISA was carried out to determine the IgG level in the sera of mice. One µg of rfsp in 100 µl of coating buffer (15 mM Na_2CO_3, 35mM $NaHCO_3$, pH: 9.5) was coated in ELISA plate wells and incubated overnight at 4°C. The wells were washed with 0.05% PBST (phosphate-buffered saline with 0.05% Tween detergent) and non-specific sites were blocked by blocking buffer (PBST+5% Skimmed Milk Powder) for 1 h at 37°C. Wells were washed with PBST and a serial dilution of antibody serum from 1:100 to 1:256000 in PBST was added to each well. The plate was kept for 2 h at 37°C and then washed with PBST. Goat Anti-Mouse IgG HRP conjugate obtained from Sigma Company (1/2000 dilution in PBST) was added to each well and incubated at 37°C for 1.5 h. After washing, 100 µl of 3, 3′, 5, 5′- tetramethylbenzidine (TMB) substrate (0.4 g/liter) was added to each well. The reaction was stopped by H_2SO_4 2M and the plate was read at 450 nm.

Detection of inactivated SARS-CoV-2 virus by serum of mice vaccinated with rfsp. 10^6 pfu of SARS-COV-2 virus, which was cult culture Vero cells and inactivated using 4% paraformaldehyde, was prepared with a concentration of 100 µg from the Pasteur Institute of Iran. After optimization *in vitro*, a concentration of 1000 ng of inactivated virus was selected for ELISA test. DEME (Dulbecco's Modified Eagle Medium) medium containing Vero cell was considered as the negative control. 1000 ng of inactivated SARS-CoV-2 virus was coated in 100 µl of coating buffer on ELISA plate and serum of mice vaccinated with rfsp was added to each well in 1: 100 dilution with PBST.

ELISA assay for investigating the immunogenicity of the rfsp as a vaccine. To investigate the potential immunogenicity of the rfsp as a vaccine in humans, we collected serum samples from 30 COVID-19 convalescent patients, with SARS-CoV-2 alpha variant from Baqiyatallah Hospital of Tehran, 30 COVID-19 convalescent patients with SARS-CoV-2 delta variant from Nabi Akram Hospital of Sistan and Baluchestan Province, and 20 healthy donors. According to the inclusion criteria of the current study, 2019 COVID-19 patients who were confirmed in terms of clinical criteria, etiological characteristics, positive RT PCR testing and were willing to cooperate in the study, were included. On the other hand, based on the exclusion criteria, those with a history of chronic diseases such as infectious diseases (chronic brucellosis, chronic hepatitis B or C, & HIV), cancers (leukemia & lymphoma), and allergic disorders were excluded. The binding of the serum antibody to

rfsp was detected with ELISA as described above. In brief, rfsp was used to coat 96-well microtiter plates. After blocking with 5% skim milk, 1:5 diluted serum was added and the plates were incubated for 1 h at room temperature, accompanied by four PBST washes. Bound antibodies were recognized with Goat Anti-human IgG antibodies conjugated with horseradish peroxidase (Sigma).

Investigation sera from immunized mice for the blocking activity of the RBD binding to ACE2 receptor. Sera from immunized animals were tested for the blocking activity of the RBD binding to ACE2 receptor by using SARS-CoV-2 Neutralizing Antibody ELISA kits (Pishtaz Teb, Iran, https://pishtazteb.com/) according to the manufacturer's protocol.

Statistical analyses. The results of ELISA for antibody responses in immunized and non-immunized groups and SARS-CoV-2 Neutralizing Antibodies were analyzed using SPSS version 24 and One-Way ANOVA. Differences were considered statistically significant if $P<0.05$.

RESULTS

Confirmation of plasmid pcNA3.1 containing rfsp. The recombinant plasmid pcNA3.1 containing rfsp was extracted (Fig. 1a). PCR was done with pcDNA 3.1 primers to ensure the rfsp gene presence in the pcNA3.1 plasmid. The 1200 bp chimeric fragment was seen on 1% agarose gel (Fig. 1b). Then recombinant Plasmid pcDNA3-rfsp was linearized with SspI enzyme (Fig. 1c).

PCR reaction to confirm CHO cell genome harboring rfsp gene. The individual clones were monitored for rfsp production during the predetermined 21 days (Fig. 1d). PCR reaction was used to analyze the colonies. After extraction of the transfected CHO cell genome, a PCR reaction was performed using specific primers of the rfsp. The PCR reaction product was evaluated on a 1% gel (Fig. 1e).

Expression of rfsp gene. The expression of recombinant protein was analyzed on 12% SDS-PAGE and desired rfsp (60 kDa) purification of the recombinant protein was performed by Ni-NTA affinity (Fig. 2a). Estimation of the purified recombinant protein by the Bradford method indicated that the concentration of the rfsp was 128 µg/ml.

Western blotting to confirm the expressed rfsp. Anti-poly His-tag antibody was used to confirm the authenticity of rfsp (60 kDa) proteins. The result indicated no reactivity in the negative control (Fig. 2b).

Determination of serum IgG titer. Blood samples were taken, and antibody titers of IgG were assessed. Repeated measurement was used to analyze the effect of the booster immunization number (Fig. 3). Indirect-ELISA indicated that the increase in antibody titer following each booster dose administration was significant ($P<0.05$).

Detection of SARS-CoV-2 virus using immunized mice sera. The findings revealed that the serum of mice immunized with rfsp was able to bind to the killed SARS-CoV-2 virus as an antigen and indirect-ELISA confirmed its significance ($P<0.05$) when compared to the control group (Fig. 4a).

Reactivity of rfsp in ELISA for SARS-CoV-2 antibody detection. The delta variant of the virus is less susceptible to bind to rfsp in sera from recovered patients compared to the alpha variant of the virus (Fig. 4b). Indirect-ELISA confirmed the significance ($P<0.05$) of the obtained result.

Characterization of sera from immunized animals. The findings indicated that the sera from immunized mice in comparison to unimmunized mice could effectively block the binding of the RBD to the ACE2 receptor in human cells (Fig. 4c). According to the kit instruction, the lower absorption indicates higher protection. Calculations show that immunized mice have 70% and unimmunized mice have 0% protective antibodies.

DISCUSSION

Among the variety of applications reported for recombinant spike protein (22) are its use in developing new treatment methods (e.g., neutralizing antibodies or other spike-targeting biologics) (23) as well as its use in clinical diagnostic devices (to check post-infection immunity) (24) or in subunit vaccines (different forms of recombinant SARS spike protein

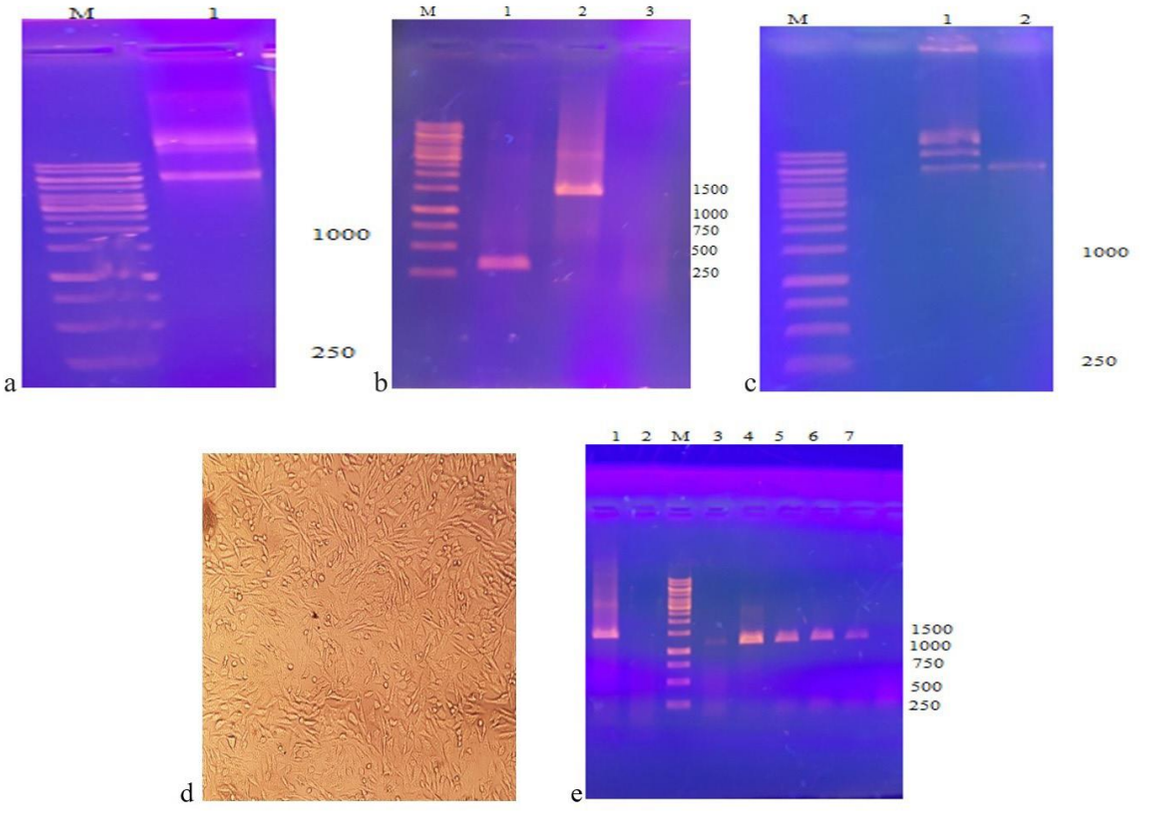

Fig. 1. Electrophoresis of pcDNA3- rfsp plasmid

a: Extracted plasmid; M, DNA marker; 1, the plasmid pcDNA3 containing rfsp gene.

b: PCR product with pcDNA 3.1 primers; M , DNA marker; 1, PCR product with pcDNA3.1; 2, PCR product with pcDNA3-rfsp; 3, negative control(PCR product with out tamplet DNA for checking contamination).

c: plasmid pcDNA3-rfsp was linearized with SspI enzyme; M, DNA marker; 1, supercoil pcDNA3-rfsp plasmid; 2, linear plasmid pcDNA3-rfsp

d: CHO cells that were transfected that grew after 21 days in a medium containing 600 µg/mL Geneticin and were able to produce rfsp

e: PCR product with rfsp primers; 1, positive control (PCR product with pcDNA3-rfsp vector); 2, Negative control (PCR product with CHO cell genome that was not transfected); M: DNA marker; 3-7, Reaction product PCR with CHO genome transfected in dilutions of 1:10, 1:50, 1: 100, 1: 200, 1: 500, respectively.

have been reported to be effective vaccines in animal models) (25, 26). The lack of post-translational modification machinery in bacteria has resulted in an immense challenge posed by recombinant eukaryotic protein production, leading to the biologically inactive protein production in this host (27). The expression system of the mammalian cells is a fairly mature eukaryotic system for recombinant protein expression. The system can stably or transiently express recombinant antigens, elevate signal synthesis, and properly fold, secrete, and glycosylate synthesized proteins (21). The expressed recombinant proteins then may retain good antigenicity, immunogenicity, and native conformation. Thereafter, the system has been currently used for expressing a wide range of heterologous proteins, namely bioactive peptides and viral structural proteins, for certain functional analysis (28). The mammalian cell system has far higher production costs and lower productivity compared with *E. coli* and the insect cell expression systems. The main advantage of this system compared to the other two expression systems is that there is a relatively greater chance of getting properly folded and glycosylated soluble proteins, which helps the expressed protein keep native conformation and adequate bioactivity (17). In this study, we

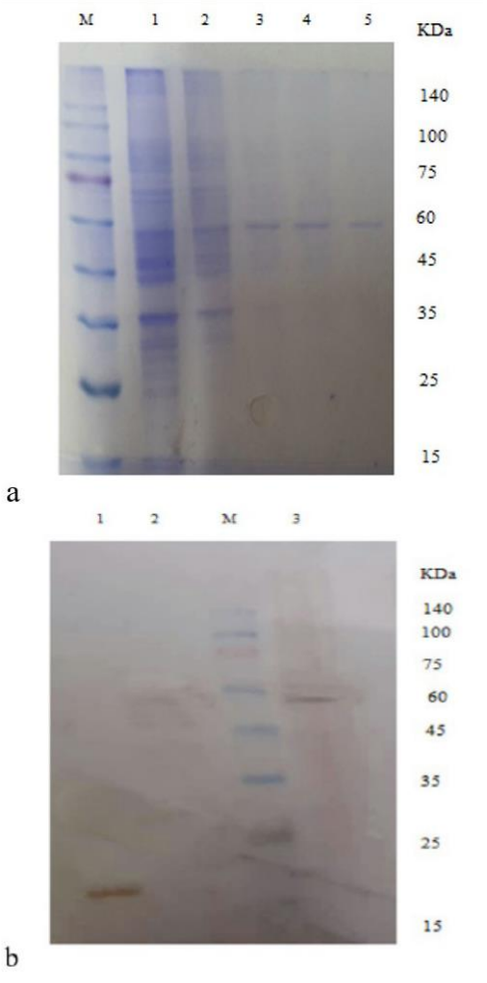

Fig. 2. a: Electrophoresis of the rfsp purified by the Ni-NTA column on 12% SDS-PAGE;1 cell lysate; 2-5, purified protein (Elution Buffer); M, protein ladder
b: Western blotting with the anti-His antibody; 1, positive control; 2, negative control; 3, rfsp; M, protein ladder

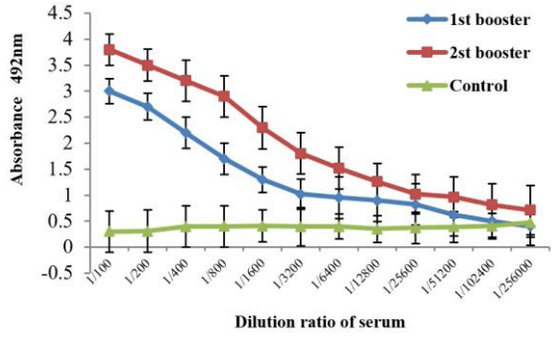

Fig. 3. The analysis of immune response type in mice immunized with rfsp. Serum-specific IgG titers were taken from immunized mice. The detection was performed by ELISA. Purified rfsp protein was applied as antigen.

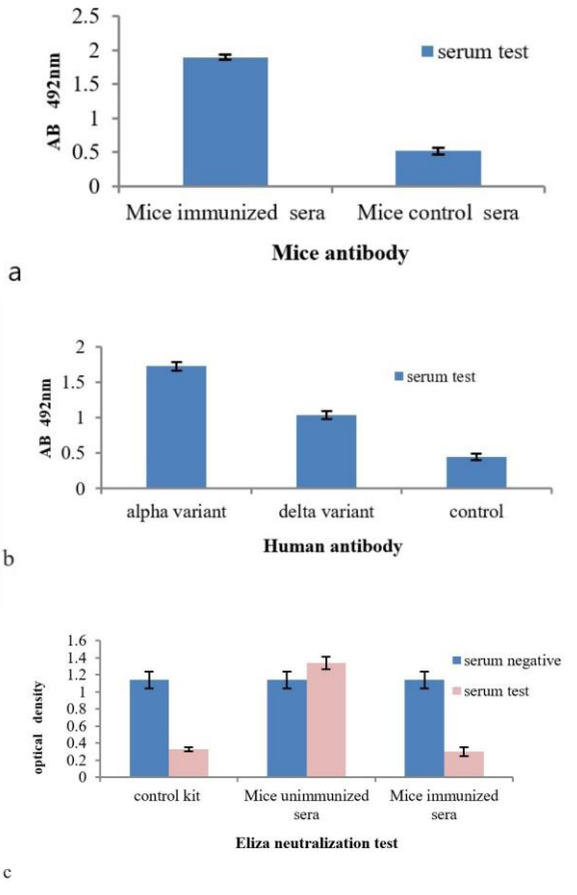

Fig. 4. Evaluation of the efficacy of epitopes rfsp
a: Detection of SARS-CoV-2 virus using sera of mice immunized with rfsp
b: Diagnosis of rfsp using serum of patients infected with SARS-CoV-2 alpha and delta variants
c: The potential of immunized mice to neutralize antibodies

aimed at designing a subunit vaccine targeting the spike protein of SARS-CoV-2, which is one of the major determinants of antigenicity and viral entry into the host cell (5, 29). We selected virus epitopes that have antigenic and neutralizing antibodies and play a key role in the mechanism of virus entry and pathogenesis. The SARS-CoV-2 spike protein has an S1 / S2 cleavage region that is broken down by host cell furin protease. This incision in the spike is essential for entry into lung cells. If antibodies are produced against this region, the furin protease will not be able to function properly, so the virus will not be able to enter the cell. According to WHO Landscape (https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines), we produce subunit vaccines from different parts of Spike.

Our DNA fragment consisted of three putative antigens RBD, S1/S2 cleavage, and FP. The SARS-CoV-2 S gene encodes 22 N-linked glycan sequons (A sequon is a sequence of consecutive amino acids in a protein that can serve as the attachment site to a polysaccharide, frequently an N-linked-Glycan) per protomer, which probably is involved in protein folding and immune elusion (6). The CHOcell was chosen as it is a suitable host for spike expression due to its post-translation and glycosylation (30). The glycosylation pattern of the spike protein is a necessary characteristic with regard to steric hindrance, chemical properties, and its potential to be a target for mutation in the future (15). Spike glycosylation not only has a protective role, but also contributes to immune elusion by covering viral polypeptide epitopes (31). Lanying Du et al. (2009) stated that the RBD expressed in *E. coli* is not as immunogenic as those expressed in mammalian and insect cells (32). As an important method, serological testing is used for the diagnosis of SARS-CoV-2 infection (33). Johari et al. (2020) found that CHO cells could be a proper host for the production of a large amount of recombinant SARS-CoV-2 trimers which can be used as antigens for mass serological testing (3). Our result showed the rfsp was detected by the sera of Cov-19 convalescent patients (Fig. 4b). Moreover, the reactivity of SARS-cov-2-killed virus with sera obtained from rfsp protein of immunized mice indicated that this protein can present similar epitopes with the viral strain (Fig. 4a). S-protein can induce protective immunity and neutralizing antibodies (nAbs) compared to other structural proteins (34). Due to the lack of access to the BSL3 laboratory, we performed a neutralization test using an ELISA neutralization kit. This kit contains a vial of human serum which has a specified amount of neutralizing antibody as positive control and negative control human serum which has no neutralizing antibody. According to the kit instruction, the lower absorption indicates higher protection. Calculations show that immunized mice have 70% and unimmunized mice have 0% protective antibodies. These antibodies in immunized mice can block the RBD and its ACE2 receptor interaction, and affect neutralization against SARS-CoV-2 (Fig.4c). If recombinant vaccines are used alone, they will mostly show low immunogenicity. Therefore, an adjuvant should be used along with a recombinant. We used complete and incomplete Freund's adjuvants and the obtained findings were comparable to the previous results. Qian H et al. (2022) reported that the endpoint of the serum antibody titer with the highest dilution was 2.1-fold higher than the optical absorbance value of the negative control (35). According to Fig. 3, the endpoint of the serum antibody titer of mice immunized with our chimeric protein was 3-fold higher than the optical absorbance value of the negative control. Konlavat Siriwattananon et al. (2021) Reported IgG antibody titer against SARS-cov-2 subunit vaccine up to 10,000 dilutions (36). Our result shows IgG antibody titer up to 25,000 dilutions. The rfsp induced the immune response, and antibody titer increased in the immunized animals compared to the control group (Fig. 3). It is suggested to evaluate cellular immunity (in addition to) in immunized mice with the recombinant rfsp protein in future studies. One of the limitations of the present study was the lack of facilities and equipment to perform the challenge test with the standard SARS-COV-2 virus.

CONCLUSION

In summary, the rfsp of SARS-CoV-2 spike protein expressed in CHO cells could induce highly potent neutralizing antibody responses. As working with coronavirus is very dangerous and needs the BSL3 laboratory, we used antibodies taken from convalescent patients to detect our chimeric protein. As a result, it can be said that this protein has the correct folding from the native protein and the antibody of the convalescent participants was able to recognize it. Successfully recognizing the recombinant rfsp indicates the presence of suitable epitopes in the chimeric protein. Moreover, rfsp has the potential to be a useful option for development of assays for serodiagnosis of SARS-CoV-2 infection. The obtained results indicate the production of the S- protein fragment as an antigen for serological ELISA test, offering a safe cost-effective tool for the detection of patients positive for SARS-CoV-2 which could consequently be a sensitive method for early detection of SARS- CoV-2 infection.

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