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Detection of SARS-CoV-2 peptide-specific antibodies in Syrian hamster serum by ELISA

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ABSTRACT

Golden Syrian hamsters are increasingly used as a permissive animal model for SARS-CoV-2 virus studies, but the lack of immunological assays and other immunological reagents for hamsters limits its full potential. Herein, we developed an ELISA method to detect antibodies specific to peptides and proteins derived from SARS-CoV-2 virus in immunized golden Syrian hamsters. Under optimized conditions, this assay quantitates antibodies specific for individual viral peptides, peptide pools, and proteins. Hence, this ELISA method allows investigators to quantitatively assess humoral immune responses at the peptide and protein levels and has potential application in the development of peptide-based vaccines to be tested in hamsters.

1. Introduction

Since its first report in December 2019 (Zhu et al., 2020), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and its associated disease, COVID-19, has triggered an unprecedented explosion of research on its pathogenesis, the development of therapeutics, prophylactics (*i.e.*, vaccines), and treatment modalities (Harrison, 2020; Le et al., 2020; Mullard, 2020). Various animal models have been explored for SARS-CoV-2 studies, of which the golden Syrian hamster (*Mesocricetus auratus*) has emerged as one of the best clinically relevant small animal models (Lakdawala and Menachery, 2020; Muñoz-Fontela et al., 2020; Pandey et al., 2021). Similar to other respiratory viruses, such as influenza virus and SARS-CoV (Roberts et al., 2005; Iwatsuki-Horimoto et al., 2018), both *in silico* study and experimental conditions have shown that the golden Syrian hamster is natively susceptible to SARS-CoV-2 infection without the need for genetic alteration of the host and/or virus (as is necessary with the murine model of disease) and develops signs of respiratory diseases similar to those observed in humans suffering from mild to moderate COVID-19. Therefore, it is a permissive model that recapitulates many of the clinical characteristics of human disease, making it an excellent animal model for SARS-CoV-2-related studies (Chan et al., 2020; Imai et al., 2020; Sia et al., 2020). However, the lack of available immunological assays (and reagents) limits the utilization of hamsters as an animal model to assess immune responses to viral infection and/or vaccination.

Enzyme-linked immunosorbent assays (ELISA) are a well-known laboratory technique used in both clinics and research laboratories for the detection of proteins, peptides, and other small molecules (Engvall and Perlmann, 1972). A key feature of ELISA lies in the specific interaction of an antibody and its ligands, which is exploited to detect the presence of either the antibody or its ligands (Lequin, 2005). An ELISA method has been optimized for the detection of antibodies against the whole SARS-CoV-2 virus in immunized hamsters (Shete et al., 2021), the SARS-CoV-2 full-length spike (S) protein, the receptor-binding domain (RBD) in mice (Bayarri-Olmos et al., 2021; Shalash et al., 2021). Unfortunately, there exists no ELISA method for the detection of peptide-specific antibodies in hamster sera following infection/vaccination with SARS-CoV-2. With the increasing use of golden Syrian hamsters in vaccine research for SARS-CoV-2, there is an urgent need for the development of an ELISA method for the detection of protein-specific and peptide-specific antibodies in hamster serum.

With respect to controlling or resolving the COVID-19 pandemic, effective vaccines are a critical tool (Randolph and Barreiro, 2020). Almost all current COVID-19 vaccines, despite different strategies and platforms, are designed using the viral S protein as the antigenic component. Although it is a logical choice of antigen, S protein is characterized by a high rate of mutation, leading to new variants with the ability to partly, or even fully, evade vaccine-induced immunity, necessitating the development of newer COVID-19 vaccines. Of the many potential vaccine strategies, peptide-based vaccines are emerging as a promising approach for broadly protective COVID-19 vaccines (Poland et al., 2021). In this study, we developed a new ELISA method to

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detect peptide-specific antibodies in hamster serum. Our data show that this new ELISA detects both protein-specific and peptide-specific antibodies in immunized hamsters, demonstrating that this ELISA is a useful method for evaluating the immunogenicity of COVID-19 vaccines, including peptide-based vaccines, in golden Syrian hamsters.

2. Material and methods

2.1. Reagents

Rabbit anti-hamster peroxidase-conjugated IgG antibody (catalog no. 207–4302) and TMB (3, 3′, 5, 5’-Tetramethylbezidine) ELISA peroxidase substrate (catalog no. TMBE-1000) were purchased from Rockland Immunochemicals, Inc. (Limerick, PA). Bovine serum albumin (BSA, catalog no. A7030) was obtained from Sigma-Aldrich (St. Louis, MO). Immulon 4 HBX 96-well plates with clear flat bottom (catalog no. 3855) were purchased from Thermo Fisher Scientific (Waltham, MA). Coronavirus nucleocapsid (N) (catalog no. 40588-V08B) and spike (S) (catalog no. 40589-V08B1) recombinant proteins were provided by Sino Biological US Inc. (Wayne, PA). SARS-CoV-2-derived peptides were synthesized and purified by the Proteomics and Peptide Synthesis Core facility at Mayo Clinic (Rochester, MN). These peptides were either predicted by an immunoinformatic approach (Crooke et al., 2020) or identified by mass spectrometry approaches using SARS-CoV-2-infected cells. However, the sequences of these peptides are under examination for potential intellectual property protection by Mayo Clinic; hence, their sequences are not provided. Note that exact sequence information is not necessary to evaluate the use of the assay. Stock solutions of these peptides were prepared at 20 mg/ml in DMSO and diluted in PBS as needed. Other reagents were at analytical grade and used as provided, unless stated otherwise.

2.2. ELISA procedure

The overall procedure for the ELISA is summarized in Fig. 1. First, SARS-CoV-2 N protein or peptides were diluted in a coating buffer (sodium bicarbonate, $NaHCO_3$, 50 mM, pH 9.6) and added to ELISA plate wells at 100 µl/well overnight at 4 °C. After discarding the coating buffer, the ELISA plate wells were blocked with 200 µl/well of BSA solution in PBS for 1 h at room temperature. After blocking, the plate wells were washed three times with 0.5% Tween (*v*/v) in PBS (PBST). Serially diluted hamster serum was added at 100 µl/well and the plate was incubated at room temperature for 2 h. Following serum incubation, the plate wells were washed three times with PBST. Rabbit anti-hamster IgG antibody conjugated with peroxidase was diluted in PBS and added to the wells at 100 µl/well. The plate was incubated at room temperature for 1 h. After incubation, the plate was washed three times with PBST. After washing, TMB substrate (100 µl) was added to each well and was followed by 30 min of incubation at room temperature. Finally, 2 M H_2SO_4 (50 µl) was added to stop the reaction. Optical density at 450 nm (OD450) was measured within 15 min following the addition of H_2SO_4.

For optimization purpose, the amount of peptide/protein used to coat each well was varied from 0.1 to 1 µg/well. Additionally, BSA solutions at 1% and 2% were used while the dilution factor of secondary antibody was optimized from 1:50,000 to 1:10,000.

2.3. Hamster immunization

Female golden Syrian hamsters (Charles River Laboratories, Wilmington, MA) were maintained in a BSL-2 containment facility under pathogen-free conditions at Mayo Clinic (Rochester, MN) in accordance with the Institutional Animal Care and Use Committees guidelines. A group of 3 hamsters was subcutaneously immunized with a two-dose regimen with a 14-day interval between vaccines. Each hamster received either 20 µg of SARS-CoV-2 N protein (Fig. 2A) or a pool of 20 peptides (20 µg each) (Fig. 4A) formulated with the 100 µg of a class C CpG (ODN2395; InvivoGen; San Diego, CA) in a water-in-oil emulsion of Complete Freund's Adjuvant (CFA). The volume ratio of protein or peptide, CFA, and CpG was kept at 1:2:1. Naïve hamsters were left unimmunized. Hamsters were euthanized at 14 days after the second dose and blood samples were collected by cardiac puncture. Hamster serum was prepared by centrifugation at 3000 ×*g* for 15 min at 4 °C and then stored at -80 °C for further analyses.

To determine if this methodology could be used to detect the presence of antibodies specific to SARS-CoV-2, hamsters ($n = 6$) were intranasally infected with 1×10^6 $TCID_{50}$ of SARS-CoV-2 virus betaCoV/USA-WA-1/2020 (Fig. 3A). Blood was sampled at day 0 (before infection), day 3, day 7 after viral infection. Hamster serum was prepared by centrifugation at 3000 ×*g* for 15 min at 4 °C and heat inactivated (70 °C for 30 min) before storing at -80 °C for further use. Hamster used in this experiment were maintained in a BSL-3 containment facility at Department of Veterinary Microbiology and Preventive Medicine, Iowa State University (Ames, Iowa) under the supervision of Dr. Bryan H. Bellaire. All SARS-CoV-2-infected hamster experiments were reviewed and approved by the Institutional Animal Care and Use Committees at Iowa State University.

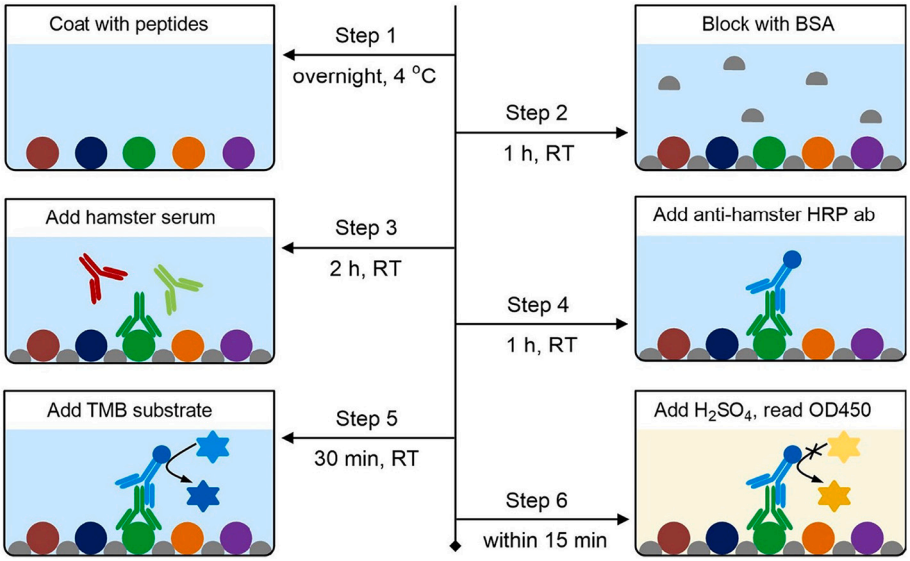

Fig. 1. Overall ELISA method. Proteins or peptides in sodium bicarbonate coating buffer were used to coat Immulon 4 HBX plate overnight at 4 °C (step 1). The plate wells were blocked with 2% BSA solution in PBS at room temperature (RT) for 1 h (step 2). After washing three times with 0.5% Tween 20 in PBS (PBST) diluted hamster serum was added (step 3). This was followed by three additional washes with PBST and the addition of diluted anti-hamster secondary antibody conjugated with HRP (step 4). After the incubation with secondary antibody, plates were washed three times with PBST to remove unbound secondary antibody. Then, TMB substrate was added and the plate was incubated at RT for 30 min (step 5). Finally, H_2SO_4 was added to stop the enzymatic reaction and optical density at 450 nm (OD450) was measured within 15 min following the addition of H_2SO_4.

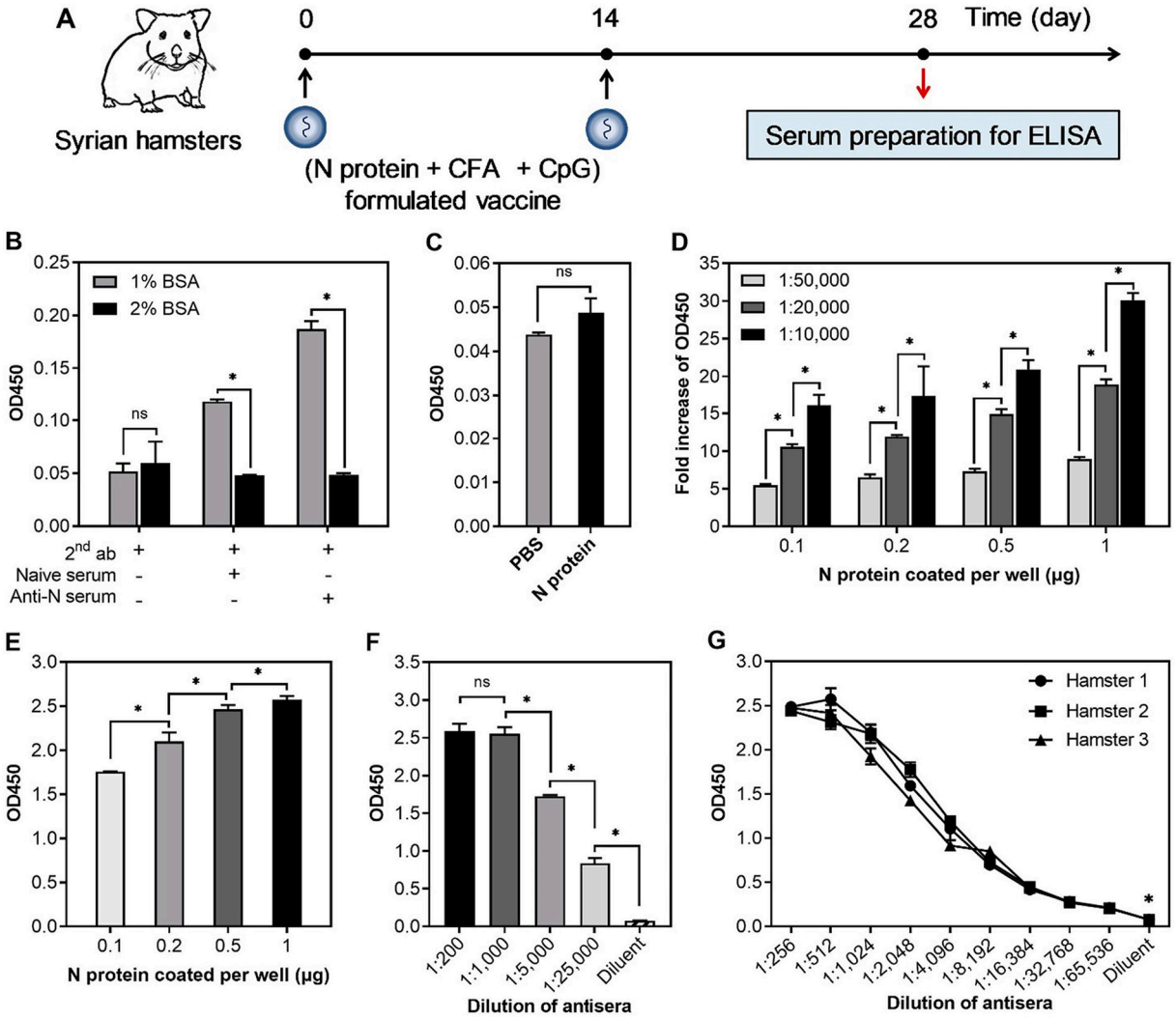

Fig. 2. Detection of anti-N antibodies in hamster serum. (A) Hamsters ($n = 3$) were immunized with 20 μg of recombinant SARS-CoV-2 N protein formulated with CpG and CFA in a 2-dose regimen with 14-day interval. Hamster serum was prepared for ELISA at 14 days after the second dose. (B) Comparison of two blocking conditions in uncoated wells. The "+" symbol indicates the addition of reactants while the "-" symbol means no reactant was added. (C) At 2% BSA blocking, there was no significant interaction detected between the N protein and the secondary antibody. (D) The plate wells were coated with N protein at 0.1, 0.2, 0.5, and 1 μg/well while the secondary antibody was diluted from 1:50,000 to 1:10,000 in PBS. Each bar indicates the fold increase of OD450 or the ratio of OD450 between anti-N serum and naïve serum. (E) The wells were coated with N protein at 0.1, 0.2, 0.5, and 1 μg/well and OD450 was compared at the 1:10,000 dilution of secondary antibody. (F) At 1 μg/well of N protein coated and 1:10,000 dilution of secondary antibody, OD450 was compared among different dilutions of pooled serum from N-protein immunized hamsters. (G) Serum from each hamster was serially diluted and anti-N antibodies were detected by using the optimized conditions. "ns" stands for nonsignificant difference while "*" indicates a statistically significant difference with $p < 0.05$. "*" at "diluent" in Fig. 2G indicates a significantly lower level of OD450 in the diluent as compared to all dilution factors of the anti-N serum. All experiments were performed in triplicate and data was shown as mean ± standard deviation (SD).

In a separate experiment, hamsters ($n = 5$) were intramuscularly immunized with 100 μl (corresponding to 6 μg) of the BNT162b2 mRNA COVID-19 vaccine (Pfizer-BioNTech) with a two-dose regimen of 28-day interval between vaccines (Fig. 3B). Hamsters were euthanized at 14 days after the second dose and blood samples were collected by cardiac puncture. Hamster serum was prepared by centrifugation at 3000 ×*g* for 15 min at 4 °C and then stored at -80 °C for further detection of anti-N and anti-S antibodies.

2.4. Statistical analysis

The differences in OD450 between different experimental conditions were compared using a 2-tailed *t*-test, with $p < 0.05$ as the threshold for significance. The coefficient of variation (CV) was calculated as: CV (%) = 100 × (SD/M), where SD is standard deviation of mean (M). The figures were plotted using GraphPad Prism software version 9.1.

3. Results

3.1. Detection of anti-N protein antibodies

Polystyrene 96-well plates (Immulon 4 HBX) with an enhanced hydrophilic binding surface (ideal for antibody sandwich assays) were used in this study. However, one of practical issues with high-binding surfaces is the direct adsorption of reactants to these surfaces, leading to high background and false positive signals. Blocking (step 2 in Fig. 1) is a critical step to prevent direct absorption. Bovine serum albumin (BSA) is a common choice of blocking agents (Xiao and Isaacs, 2012). In an initial experiment, the uncoated wells were blocked with BSA (step 2 in Fig. 1) then the secondary antibody (step 4 in Fig. 1) and other reactants from step 4 onwards were added. There was no significant difference between 1% and 2% BSA (Fig. 2B), suggesting that 1% BSA was sufficient to block the well surface from direct adsorption of secondary

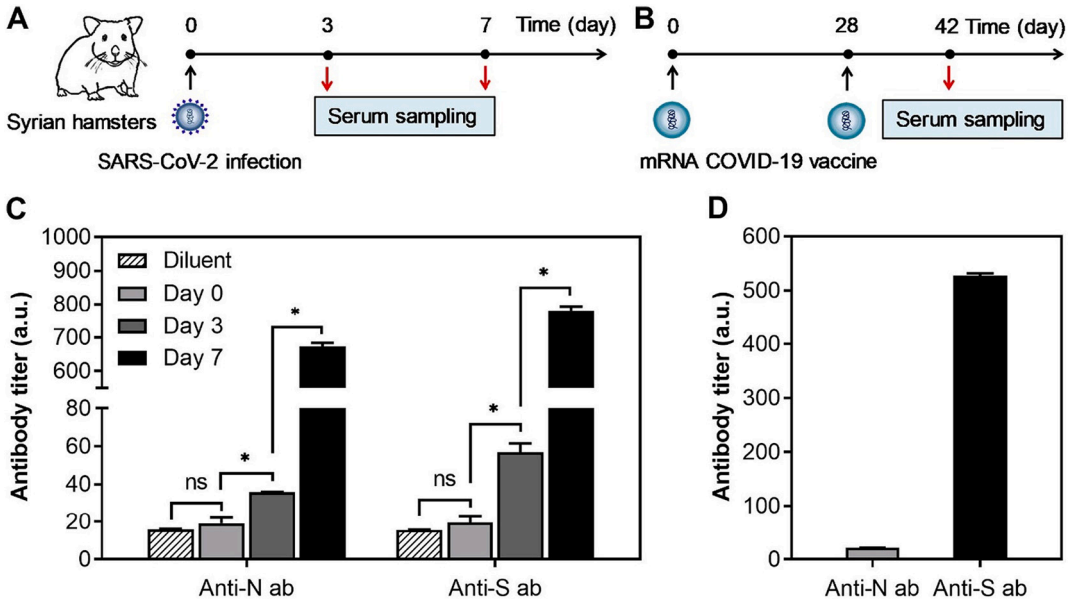

Fig. 3. Detection of SARS-CoV-2-specific antibodies in SARS-CoV-2-infected and mRNA vaccine-immunized hamsters. (A) Hamsters ($n = 6$) were intranasally infected with 1×10^6 $TCID_{50}$ of SARS-CoV-2 virus. Hamster serum was sampled at Day 0 (before infection), Day 3, and Day 7 after infection. (B) Hamsters ($n = 5$) were immunized with 2 doses of BNT162b2 mRNA COVID-19 vaccine. Hamster serum was sampled at 14 days after the second dose. (C) Detection of SARS-CoV-2-specific antibodies in pooled sera from infected hamsters. The ELISA wells were coated with either SARS-CoV-2 recombinant N or S protein. Pooled hamster sera sampled at different timepoints were diluted (1:250) in PBS. In "diluent" condition, PBS, instead of hamster serum, was added to the wells. (D) Detection of SARS-CoV-2-specific antibodies in mRNA-immunized hamsters. The plate wells were coated with either SARS-CoV-2 recombinant N or S protein and SARS-CoV-2-specific antibodies were detected under the same previously optimized conditions. Pooled hamster sera were diluted (1:250) in PBS. All experiments in (C) and (D) were performed in quadruplicate and the data was shown as mean ± standard deviation (SD).

antibody. However, given the same experimental conditions, but with the introduction of naïve or anti-N hamster sera (step 3 in Fig. 1), there was a significant increase in OD450 detected with 1% BSA as compared to background level with 2% BSA (Fig. 2B). Hence, blocking with 2% BSA was utilized for the remaining experiments.

To check if there was any non-specific interaction between coated proteins and secondary antibody, the plate was coated with SARS-CoV-2 N protein (1 μg/well) overnight at 4 °C (step 1 in Fig. 1). As a negative control, PBS was used in the place of N protein solution. After coating, all other steps, except for step 3 of adding hamster serum, were carried out. Without adding hamster serum at step 3, OD450 remained at background intensity, indicating that there was no significant non-specific interaction between the secondary antibody and N protein-coated wells (Fig. 2C).

Next, the dilution factor of anti-hamster secondary antibody was optimized. The dilution factor recommended by the manufacturer was from 1:10,000 to 1:50,000. We found that the OD450 increased with the increasing amounts of the secondary antibody across all levels of N protein coated and that a dilution of 1:10,000 resulted in the largest increase (>15-fold) in OD450, as compared to background signal (Fig. 2D). The antibody was also tested at a 1:5000 dilution; however, the OD450 was beyond the limit of detection of the UV–Vis spectrometer (data not shown). We also found that the background signals increased with the increasing amount of secondary antibody. We therefore selected a 1:10,000 dilution of secondary antibody for the next steps.

We varied the amount of N protein coated on each well and found that increasing the amount of N proteins coated on each well increased OD450 (Fig. 2D). Coating each well with 1 μg of N protein resulted in the highest OD450 (Fig. 2E). Coating the wells with more than 1 μg/well of N protein did not lead to any significant increase of OD450. Therefore, 1 μg/well was selected as optimal amount of protein for well coating.

Under optimized conditions, this ELISA method was used to detect anti-N antibodies in hamsters immunized with N protein. In pooled hamster serum, there was no significant difference in OD450 between the 1:200 and 1:1000 dilutions (Fig. 2F). However, the OD450 decreased with the increase in dilution factors from 1:1000 to 1:25,000 (Fig. 2F). Serum from each immunized hamster was serially diluted and anti-N antibodies were detected in each hamster (Fig. 2G). Surprisingly, anti-N antibodies could be detected even at 1:65,536 dilution and there was no significant change in antibody level among 3 hamsters (Fig. 2G). OD450 measured at all dilution factors of anti-N serum was significantly higher than that of the diluent (Fig. 2F and G). At optimized conditions, an average coefficient of variation (CV) of 3.05% was calculated across all dilutions of anti-N sera in 3 hamsters.

3.2. Detection of SARS-CoV-2-specific antibodies

After the optimization with N protein (Fig. 2), we explored the capability of the assay to detect the antibodies specific to SARS-CoV-2 proteins in SARS-CoV-2-infected hamsters (Fig. 3A) and in hamsters immunized with mRNA vaccine (Fig. 3B). In SARS-CoV-2-infected hamsters, anti-N and anti-S antibodies were undetected (signal equivalent to background levels) in pooled serum at Day 0 (Fig. 3C). However, SARS-CoV-2 protein-specific antibodies were detected as early as Day 3 after infection with readings of 35.64 (arbitrary unit, a.u.) and 56.83 (a.u.) for anti-N and anti-S antibodies, respectively. At Day 7 after infection, the levels of anti-N and anti-S antibodies increased markedly to 673.16 and 781.16 (a.u.), respectively (Fig. 3C).

We further explored whether or not the assay could be used to detect SARS-CoV-2-specific antibodies in hamsters immunized with BNT162b2 mRNA COVID-19 vaccine (Pfizer-BioNTech). As shown in Fig. 3D, while anti-N antibodies were detected at a background level of 21.74 (a.u.), anti-S antibodies were detected at an elevated level of 527.93 (a.u.). These results confirmed the capability of the assay in detecting the presence of SARS-CoV-2-specific antibodies in both SARS-CoV-2-infected hamsters and hamsters immunized with mRNA vaccine.

3.3. Detection of antibodies specific to SARS-CoV-2-derived peptides

Having optimized with the N protein (Fig. 2) and confirmed the

detection of anti-S, anti-N antibodies in SARS-CoV-2-infected and mRNA-immunized hamsters (Fig. 3), we determined if this assay could be used to detect peptide-specific antibodies in hamster sera. Hamsters were immunized with a pool of 20 SARS-CoV-2-derived peptides. These peptides were derived from N, S, open reading frame (ORF), and membrane (M) proteins of SARS-CoV-2 virus. These peptides varied in length from 9 to 24 amino acids, had varying degrees of hydrophobicity and a range of isoelectric points. Peptides were mixed with CFA, a well-known water-in-oil emulsion, and ODN2395 (InvivoGen), a CpG class C TLR-9 agonist. Using peptides predicted to be immunogenic, the peptide vaccine formulation served as a useful model to validate the peptide-specific antibody detection capacity of this ELISA method.

The plate wells were coated with 1 μg/well of 20-peptides pool (0.05 μg of each peptide/well) and peptide-specific antibodies were detected using this ELISA method at optimized conditions. Similar to N protein (Fig. 2C), there were no significant non-specific interactions between the secondary antibody and coated peptides (data not shown). In pooled hamster serum, peptide-specific antibodies were detected at a titer of 363 (a.u.) in immunized hamsters, as compared to a titer of 12 (a.u.) in naïve hamsters (Fig. 4B). We subsequently coated the plate wells with 1 μg/well of individual peptides and explored if this assay could be adapted to detect the presence of antibodies specific for each peptide. In pooled serum, OD450 signals were detected for 7 peptides (peptide ID: 12, 13, 14, 15, 17, 18, 19) at levels significantly greater than the background signal observed in naïve hamsters, indicating that these 7 peptides induced the generation of antibodies following vaccination (Fig. 4C). An average coefficient of variation (CV) of 7.36% was calculated in serum pool for 20 peptides.

We also evaluated the ability of the assay to differentiate between antibody responses in each hamster immunized with the peptide pool. Consistently, 13 non-responsive peptides (peptide ID: 1–11, 16, 20) did not show any evidence of inducing the generation of antibodies in any of the 3 hamsters (Fig. 4D). In contrast, there were variations in antibody response between the hamsters with the 7 responsive peptides. Only peptide 17 induced a comparable antibody response across 3 hamsters. Peptides 12, 14, and 15 induced antibody responses in 2 of the 3 hamsters, while peptides 13, 18, and 19 only induced antibody responses in 1 of the 3 hamsters (Fig. 4D). The assay was able to detect subtle differences in antibody response between inbred, but not genetically identical hamsters.

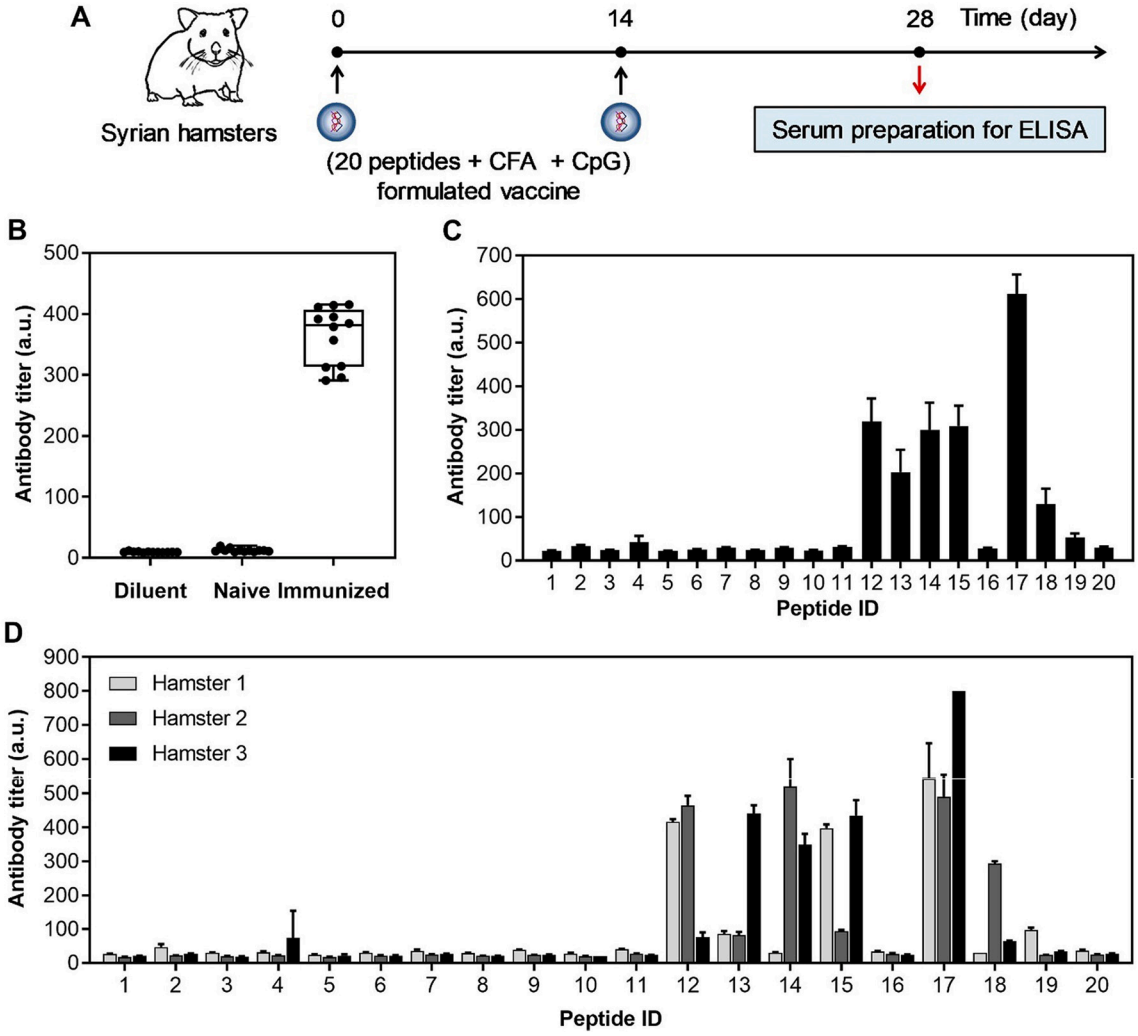

Fig. 4. Detection of SARS-CoV-2 peptide-specific antibodies in hamster serum. (A) Hamsters (n = 3) were immunized with a pool of 20 peptides (20 μg each) formulated with CpG and CFA in a 2-dose regimen with 14-day interval. Hamster serum was prepared for ELISA at 14 days after the second dose. (B) Detection of peptide-specific antibodies using a pool of 20 peptides to coat ELISA wells. Serum from each hamster of naïve or immunized group was diluted (1:200) in PBS and the measurement was performed in quadruplicate. In "diluent" condition, PBS, instead of hamster serum, was added to the wells. (C) Detection of peptide-specific antibodies individually in pooled serum. The plate wells were coated with individual peptides and peptide-specific antibodies were detected under optimized conditions. (D) Detection of peptide-specific antibodies in serum of each hamster. All experiments in (C) and (D) were performed in quadruplicate and the data was shown as mean ± standard deviation (SD).

4. Discussion

In this study, we optimized experimental conditions for an ELISA method to detect peptide-specific antibodies in immunized golden Syrian hamsters. Under optimized conditions, this assay could detect antibodies specific to both peptide pool and individual peptides, demonstrating its potential application in the development of peptide-based vaccine for SARS-CoV-2. The low coefficient of variations (CV) of <7.5% suggest a high level of precision of this assay.

Non-specific binding of reactants to the surface of the plate wells is the main source of high noise background and false positive results in ELISA assays. Numerous blocking agents have been evaluated for their abilities to prevent this non-specific binding (Péterfi and Kocsis, 2000; Xiao and Isaacs, 2012; Waritani et al., 2017), but BSA is one of the most efficient blocking agents and was used in this study. Investigators wishing to use other blocking agents should optimize those conditions before using this protocol. Although blocking with 1% BSA was sufficient to prevent the direct adsorption of secondary antibody onto the surface of the plate wells (Fig. 2B), it did not completely prevent direct absorption of the antibodies in hamster serum (Fig. 2B), resulting in increased background. It was noted that with 1% BSA, OD450 detected in anti-N serum was higher than that in naïve serum (Fig. 2B), probably due to a higher amount of total IgG in anti-N serum than in naïve serum. At 2% BSA blocking, we confirmed that there were no significant non-specific interactions between antibody-containing reactants and the plate wells, or between protein coated wells and the secondary antibody reagent (Fig. 2B and C). It was noteworthy that the OD450 in the wells coated with 0.5 μg/well of N protein was 96% of that in the wells coated with 1 μg/well (Fig. 2E). Hence, coating with 0.5 μg/well may not result in a significant loss of OD450 if antigen quantities are limited.

At optimized conditions, there was no significant difference in OD450 between 1:200 and 1:1000 dilution of hamster pooled serum, suggesting a high titer of antibodies saturating the detecting capacity of reagents (Fig. 2F). However, the difference in OD450 at higher dilution levels showed the potential of this ELISA method as a platform to screen the humoral response to protein-based or peptide-based vaccines. The effect of adjuvants, for example, on vaccine immunogenicity can easily be measured by immunizing with different adjuvants and comparing antibody titers at a fixed serum dilution. The potential of this assay was further confirmed by its capability of detecting anti-N and anti-S antibodies as early as 3 days after viral infection in SARS-COV-2-infected hamsters (Fig. 3C). Only anti-S antibodies were detected in hamsters immunized with mRNA vaccine (Fig. 3D), reflecting the intrinsic characteristic of its S protein-encoded mRNA sequence.

Although immunized hamsters developed a high antibody titer against a pool of 20 peptides (Fig. 4B), the response was driven by antibodies specific to 7 of the 20 selected peptides (Fig. 4C). These results indicated the difference in immunogenic properties of these peptides and demonstrated the ability of our ELISA method to measure the immunogenicity of peptides. We noted that N protein induced extremely high level of antibodies (Fig. 2G) as compared to the peptide pool (Fig. 4). These results have important implications for rational design of new generations of COVID-19 vaccines since N protein was found to induce SARS-CoV-2 protective immunity in a S-independent manner (Matchett et al., 2021). Newer COVID-19 vaccines may incorporate antigens such as N protein in addition to the S protein found in current vaccines.

Our results revealed variations in the antibody response to specific peptides in each hamster, potentially due to minor variations in immunization technique or slight differences in genetics between hamsters. Nevertheless, our method is capable of detecting differences in antibody titers in individual vaccine recipients and should prove useful in comparing immunogenicity between experimental animal groups (Fig. 4D). Although we optimized the experimental conditions to detect peptide-specific antibodies in hamsters, this ELISA method could be adapted to detect peptide-specific antibodies in mouse, human or any other species by switching secondary antibody reagents.

5. Conclusion

In conclusion, we have developed an ELISA method for the detection of SARS-CoV-2 protein- and peptide-specific antibodies in hamsters. At optimized conditions, this method demonstrated its ability to detect antibodies specific to SARS-CoV-2 and SARS-CoV-2-derived peptides. This assay was capable of detecting the generation of SARS-CoV-2-specific antibodies in both infected and mRNA vaccine-immunized hamsters. It was also able to discriminate inter-individual variation of immune responses in vaccinated hamsters. Although this ELISA method was optimized for hamsters, it could easily be adapted to other species by replacing the secondary reagents with reagents specific for that species. We conclude that this ELISA method expands the array of analytical tools available to study immune responses in hamsters and is therefore a useful method to assist in the development of protein-based and/or peptide-based COVID-19 vaccines.

Acknowledgement

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Declaration of Competing Interest

Dr. Poland is the chair of a Safety Evaluation Committee for novel investigational vaccine trials being conducted by Merck Research Laboratories. Dr. Poland offers consultative advice on vaccine development to Merck & Co. Inc., Avianax, Adjuvance, Valneva, Medicago, Sanofi Pasteur, GlaxoSmithKline, and Emergent Biosolutions. Drs. Poland and Ovsyannikova hold three patents related to measles and vaccinia peptide research. Dr. Kennedy holds a patent on vaccinia peptide research. Dr. Kennedy has received funding from Merck Research Laboratories to study waning immunity to measles and mumps after immunization with the MMR-II® vaccine. Drs. Poland, Kennedy, and Ovsyannikova have received grant funding from ICW Ventures for preclinical studies on a peptide-based COVID-19 vaccine. This research has been reviewed by the Mayo Clinic Conflict of Interest Review Board and was conducted in compliance with Mayo Clinic Conflict of Interest policies.

References

Bayarri-Olmos, R., Idorn, M., Rosbjerg, A., Pérez-Alós, L., Hansen, C.B., Johnsen, L.B., Helgstrand, C., Zosel, F., Bjelke, J.R., Öberg, F.K., Søgaard, M., Paludan, S.R., Bak-Thomsen, T., Jardine, J.G., Skjoedt, M.O., Garred, P., 2021. SARS-CoV-2 neutralizing antibody responses towards full-length spike protein and the receptor-binding domain. J. Immunol. 207, 878–887.

Chan, J.F.-W., Zhang, A.J., Yuan, S., Poon, V.K.-M., Chan, C.C.-S., Lee, A.C.-Y., Chan, W.-M., Fan, Z., Tsoi, H.-W., Wen, L., 2020. Simulation of the clinical and pathological manifestations of coronavirus disease 2019 (COVID-19) in a golden Syrian hamster model: implications for disease pathogenesis and transmissibility. Clin. Infect. Dis. 71, 2428–2446.

Crooke, S.N., Ovsyannikova, I.G., Kennedy, R.B., Poland, G.A., 2020. Immunoinformatic identification of B cell and T cell epitopes in the SARS-CoV-2 proteome. Sci. Rep. 10, 1–15.

Engvall, E., Perlmann, P., 1972. Enzyme-linked immunosorbent assay, ELISA: III. Quantitation of specific antibodies by enzyme-labeled anti-immunoglobulin in antigen-coated tubes. J. Immunol. 109, 129–135.

Harrison, C., 2020. Coronavirus puts drug repurposing on the fast track. Nat. Biotechnol. 38, 379–381.

Imai, M., Iwatsuki-Horimoto, K., Hatta, M., Loeber, S., Halfmann, P.J., Nakajima, N., Watanabe, T., Ujie, M., Takahashi, K., Ito, M., 2020. Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. Proc. Natl. Acad. Sci. 117, 16587–16595.

Iwatsuki-Horimoto, K., Nakajima, N., Ichiko, Y., Sakai-Tagawa, Y., Noda, T., Hasegawa, H., Kawaoka, Y., 2018. Syrian hamster as an animal model for the study of human influenza virus infection. J. Virol. 92, e01693-17.

Lakdawala, S.S., Menachery, V.D., 2020. The search for a COVID-19 animal model. Science 368, 942–943.
Le, T.T., Andreadakis, Z., Kumar, A., Román, R.G., Tollefsen, S., Saville, M., Mayhew, S., 2020. The COVID-19 vaccine development landscape. Nat. Rev. Drug Discov. 19, 305–306.
Lequin, R.M., 2005. Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA). Clin. Chem. 51, 2415–2418.
Matchett, W.E., Joag, V., Stolley, J.M., Shepherd, F.K., Quarnstrom, C.F., Mickelson, C. K., Wijeyesinghe, S., Soerens, A.G., Becker, S., Thiede, J.M., Weyu, E., O'Flanagan, S. D., Walter, J.A., Vu, M.N., Menachery, V.D., Bold, T.D., Vezys, V., Jenkins, M.K., Langlois, R.A., Masopust, D., 2021. Cutting edge: nucleocapsid vaccine elicits spike-independent SARS-CoV-2 protective immunity. J. Immunol. 207, 376–379.
Mullard, A., 2020. COVID-19 vaccine development pipeline gears up. Lancet 395, 1751–1752.
Muñoz-Fontela, C., Dowling, W.E., Funnell, S.G., Gsell, P.-S., Riveros-Balta, A.X., Albrecht, R.A., Andersen, H., Baric, R.S., Carroll, M.W., Cavaleri, M., 2020. Animal models for COVID-19. Nature 586, 509–515.
Pandey, K., Acharya, A., Mohan, M., Ng, C.L., Reid, S.P., Byrareddy, S.N., 2021. Animal models for SARS-CoV-2 research: a comprehensive literature review. Transbound. Emerg. Dis. 68, 1868–1885.
Péterfi, Z., Kocsis, B., 2000. Comparison of blocking agents for an ELISA for LPS. J. Immunoass. 21, 341–354.
Poland, G.A., Ovsyannikova, I.G., Kennedy, R.B., 2021. The need for broadly protective COVID-19 vaccines: beyond S-only approaches. Vaccine 39, 4239–4241.
Randolph, H.E., Barreiro, L.B., 2020. Herd immunity: understanding COVID-19. Immunity 52, 737–741.
Roberts, A., Vogel, L., Guarner, J., Hayes, N., Murphy, B., Zaki, S., Subbarao, K., 2005. Severe acute respiratory syndrome coronavirus infection of golden Syrian hamsters. J. Virol. 79, 503–511.
Shalash, A.O., Azuar, A., Madge, H.Y.R., Modhiran, N., Amarilla, A.A., Liang, B., Khromykh, A.A., Watterson, D., Young, P.R., Toth, I., Skwarczynski, M., 2021. Detection and quantification of SARS-CoV-2 receptor binding domain neutralization by a sensitive competitive ELISA assay. Vaccines (Basel) 9.
Shete, A., Mohandas, S., Jain, R., Yadav, P.D., 2021. A qualitative IgG ELISA for detection of SARS-CoV-2-specific antibodies in Syrian hamster serum samples. STAR Protocols 2, 100573.
Sia, S.F., Yan, L.-M., Chin, A.W., Fung, K., Choy, K.-T., Wong, A.Y., Kaewpreedee, P., Perera, R.A., Poon, L.L., Nicholls, J.M., 2020. Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. Nature 583, 834–838.
Waritani, T., Chang, J., McKinney, B., Terato, K., 2017. An ELISA protocol to improve the accuracy and reliability of serological antibody assays. MethodsX 4, 153–165.
Xiao, Y., Isaacs, S.N., 2012. Enzyme-linked immunosorbent assay (ELISA) and blocking with bovine serum albumin (BSA)—not all BSAs are alike. J. Immunol. Methods 384, 148–151.
Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R., Niu, P., Zhan, F., Ma, X., Wang, D., Xu, W., Wu, G., Gao, G.F., Tan, W., 2020. A novel coronavirus from patients with pneumonia in China, 2019. N. Engl. J. Med. 382, 727–733.