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Development of SARS-CoV-2 variant protein microarray for profiling humoral immunity in vaccinated subjects

Tzong-Shiann Ho [a,b,c], Pin-Xian Du [d], Wen-Yu Su [d], Harvey M. Santos [d,e], Ya-Lan Lin [a], Yi-Yu Chou [f], Batuhan Birol Keskin [d], Chi Ho Pau [d], Guan-Da Syu [d,g,h,*]

[a] Department of Pediatrics, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, 701, Taiwan, ROC
[b] Center of Infectious Disease and Signaling Research, National Cheng Kung University, Tainan, 701, Taiwan, ROC
[c] Department of Pediatrics, Tainan Hospital, Ministry of Health and Welfare, Tainan, 700, Taiwan, ROC
[d] Department of Biotechnology and Bioindustry Sciences, National Cheng Kung University, Tainan, 701, Taiwan, ROC
[e] School of Chemical, Biological and Materials Engineering and Sciences, Mapúa University, Intramuros, Manila, 1002, Philippines
[f] Department of Nursing, Kaohsiung Armed Forces General Hospital, Kaohsiung, 802, Taiwan, ROC
[g] International Center for Wound Repair and Regeneration, National Cheng Kung University, Tainan, 701, Taiwan, ROC
[h] Research Center of Excellence in Regenerative Medicine, National Cheng Kung University, Tainan, 701, Taiwan, ROC

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ABSTRACT

SARS-CoV-2 is quickly evolving from wild-type to many variants and spreading around the globe. Since many people have been vaccinated with various types of vaccines, it is crucial to develop a high throughput platform for measuring the antibody responses and surrogate neutralizing activities against multiple SARS-CoV-2 variants. To meet this need, the present study developed a SARS-CoV-2 variant (CoVariant) array which consists of the extracellular domain of spike variants, e.g., wild-type, D614G, B.1.1.7, B.1.351, P.1, B.1.617, B.1.617.1, B.1.617.2, and B.1.617.3. A surrogate virus neutralization on the CoVariant array was established to quantify the bindings of antibody and host receptor ACE2 simultaneously to spike variants. By using a chimeric anti-spike antibody, we demonstrated a broad binding spectrum of antibodies while inhibiting the bindings of ACE2 to spike variants. To monitor the humoral immunities after vaccination, we collected serums from unvaccinated, partial, or fully vaccinated individuals with either mRNA-1273 or AZD1222 (ChAdOx1). The results showed partial vaccination increased the surrogate neutralization against all the mutants while full vaccination boosted the most. Although IgG, IgA, and IgM isotypes correlated with surrogate neutralizing activities, they behave differently throughout the vaccination processes. Overall, this study developed CoVariant arrays and assays for profiling the humoral responses which are useful for immune assessment, vaccine research, and drug development.

1. Introduction

The pandemic of SARS-CoV-2 is almost 2 years since the initial report of wild-type virus from Wuhan, China towards the end of 2019. Due to the high mutation rates of the RNA virus, several SARS-CoV-2 variants are circulating throughout the world which include alpha, beta, gamma, delta, and omicron. To date, there are many vaccines authorized for emergency use, e.g., mRNA-1273, BNT162b2, AZD1222, JNJ-78436735, and CoronaVac. However, they are all designed using the wild-type strain. The advent of SARS-CoV-2 mutations raises concerns regarding the long-term efficacy of existing vaccines. The possibility of vaccination escape, in which vaccine-generated immunity is insufficient to give disease protection, is a concern. The existence of convalescent or vaccine-mediated immunity, together with high viral transmission, may accelerate the emergence of escape mutants. Although *in vitro* findings demonstrate a decrease in neutralizing antibody titers, the efficacy of available spike-based vaccines against the Alpha (B.1.1.7) variant of concern (VOC) does not appear to be jeopardized (Emary et al., 2021; Hall et al., 2021). So, to be able to catch up with the viral mutations in vaccine development is extremely difficult because the research, development, and clinical trial are time-consuming and expensive. Thus, the alternative strategy is to systematically evaluate the protective

Abbreviations: CoVariant, SARS-CoV-2 variant; ACE2, Angiotensin-converting enzyme 2.

* Corresponding author. Department of Biotechnology and Bioindustry Sciences, No.1, University Road, Room 89A07, Tainan City, 701, Taiwan, ROC.
E-mail address: guanda@gs.ncku.edu.tw (G.-D. Syu).

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efficacy of the current vaccines against SARS-CoV-2 variants.

The majority of SARS-CoV-2 vaccines employ three strategies: mRNA, viral vector, and inactivated virus platforms. Moderna's mRNA-1273 uses lipid nanoparticle delivery of mRNA expressing a prefusion stabilized version of spike protein obtained from SARS-CoV-2 isolates from Wuhan, China, early in the outbreak. AZD1222, which is developed at Oxford University and comprises the SARS-CoV-2 structural surface glycoprotein antigen, spike protein gene in a replication-deficient chimp adenoviral vector ChAdOx1. CoronaVac and BBIBP-CorV are two inactivated virus vaccines developed by the Chinese company Sinovac and Sinopharm, respectively. Nevertheless, the sudden rise of new circulating variants has prompted serious doubts concerning the spatial and temporal effects of these vaccines.

To evaluate the vaccine protection against ongoing variants, it is needed to establish a multiplexed platform for evaluating immune responses. The protein microarray platform is highly suited because it can immobilize multiple antigens and profile humoral immunity (Syu et al., 2020). Our group and others have developed different types of protein microarrays to profile the serum antibodies in COVID-19 patients (de Assis et al., 2021; Du et al., 2021; Jiang et al., 2020) and after vaccination with BBIBP-CorV (Ma et al., 2021). In addition to serum antibodies, neutralizing antibodies is the most important component for blocking viral entry. Until now, there is no existing platform that has multiple variant antigens nor measuring the neutralizing antibody against multiple variants. In this study, we developed a multiplexed SARS-CoV-2 Variant (CoVariant) protein array by immobilizing wild-type and eight spike variants on a slide. By incubating with anti-spike and ACE2, the CoVariant can simultaneously detect the quantity of antibody and surrogate neutralizing activity on each spike protein variant in a single assay. In addition, sera from cohorts of patients who received one or two doses of the mRNA 1273 (Moderna) or AZD1222 (AstraZeneca) vaccine against SARS-CoV-2 and its variants were used to assess the surrogate neutralization and antibody profiles after vaccination.

2. Results

2.1. Fabrication of CoVariant protein microarray

To develop the CoVariant protein microarray, we focused on the wild-type and eight common variants of SARS-CoV-2. We selected the 6x His-tagged extracellular domain (ECD) of spike protein to maintain the

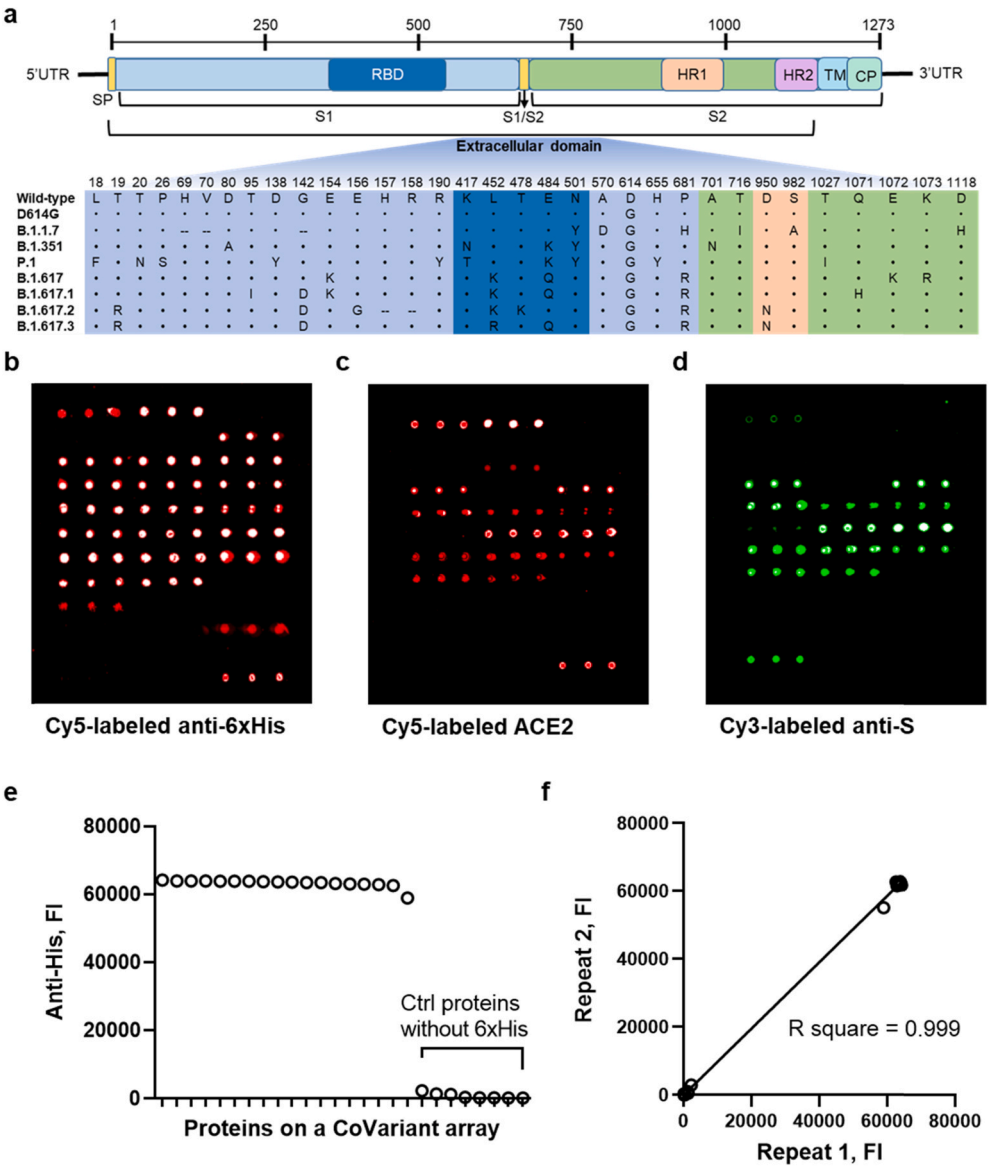

Fig. 1. Design and quality control of CoVariant protein array.
The CoVariant protein array contained the extracellular domain (ECD) of spike proteins and printed in triplicates on the array. **a** The amino acid sequences of the ECD of spike proteins from wild type SARS-CoV-2 and their variants, including D614G, B.1.1.7, B.1.351, P.1, B.1.617, B.1.617.1, B.1.617.2, and B.1.617.3. SP, signal peptide. HR, heptad repeat. TM, transmembrane domain. CP, cytoplasmic domain. **b** Fluorescence staining of 6x His to visualize the his-tagged proteins that were immobilized on the CoVariant protein array. **c** Fluorescence staining of ACE2 to show the receptor binding to the various spike ECDs. **d** Cy3-labeled pan anti-S staining to visualize the spike proteins. **e** Quantification of anti-6x His signals. **f** Reproducibility of two anti-6x His assays.

optimal antigen integrity and conformation (Fig. 1a). Moreover, ECD contained both N-terminal domain and receptor binding domain which were important in the ACE2 interactions (Liu et al., 2020; Zhang et al., 2021). Wild-type and variant spike proteins along with some control proteins were printed in triplicates on the aldehyde-coated slides and formed the most comprehensive CoVariant protein microarray. The CoVariant protein arrays were quality checked for protein immobilization, reproducibility, and protein functions. The protein immobilization was confirmed by bright signals of anti-His and anti-S staining (Fig. 1b, d, 1e). The reproducibility was evaluated by two independent anti-His stainings and showed a 0.999 r square value (Fig. 1f). The spike proteins on the array were properly folded and functional by staining with ACE2 (Fig. 1c).

2.2. Dual quantification of antibody and surrogate neutralizing activity

Given that the binding of receptor-binding domain (RBD) to the receptor ACE2 is crucial for virus entry, we measured the neutralizing activity via surrogate method as an alternative for plaque reduction assay (Tan et al., 2020). Since the spike proteins on the CoVariant protein array were functional, we aimed to quantify both antibody and ACE2 bindings using two separate fluorescence signals (Fig. 2a). As a

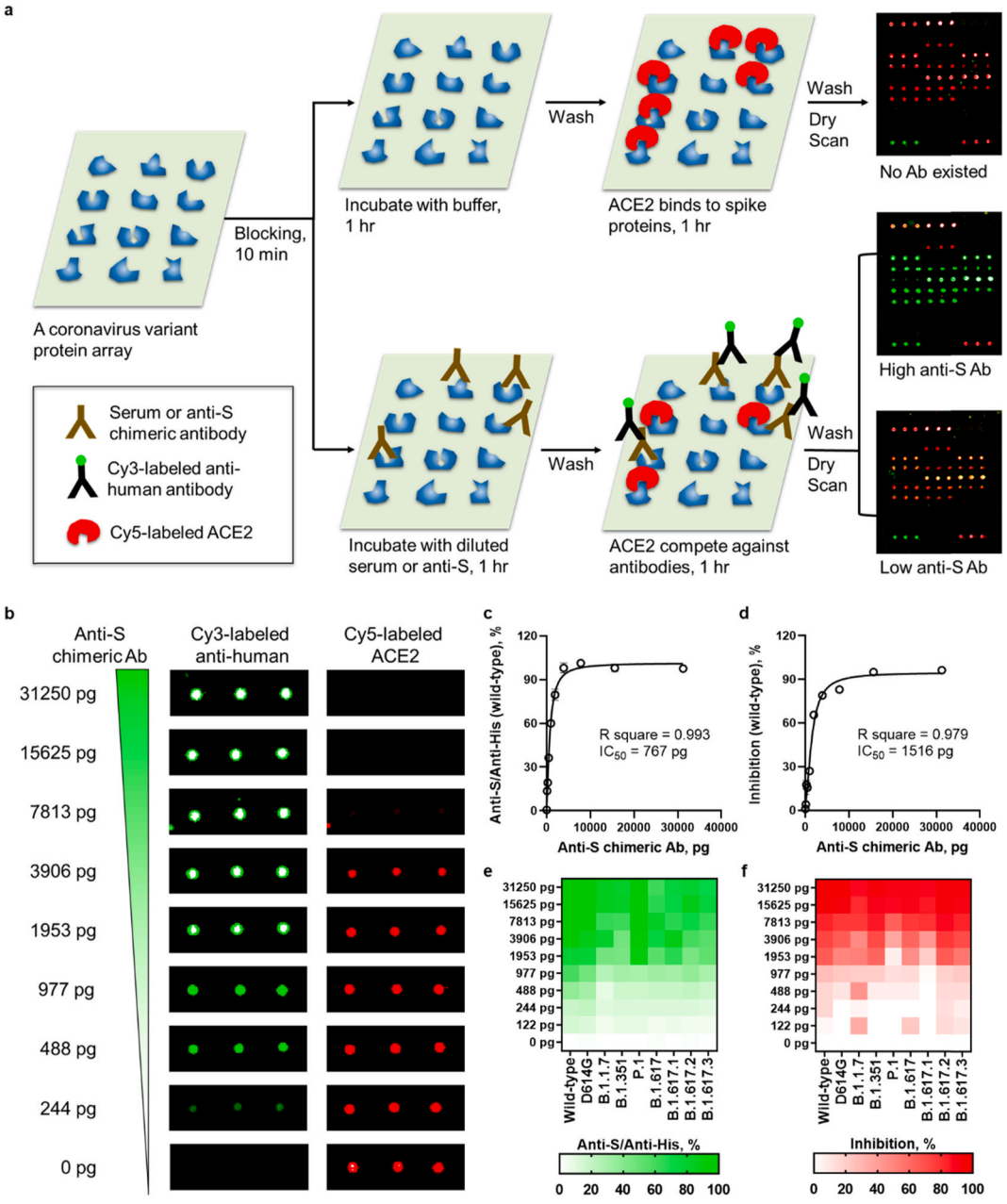

Fig. 2. High throughput detection and quantification of antibodies and surrogate neutralizing activities by using CoVariant protein array.
An anti-S antibody was used to as a proof of concept. **a** Assay procedures to simultaneously quantify antibodies and surrogate neutralizing activities. The antibody amounts from either serum or anti-S were quantified by Cy3-labeled anti-human antibody. The surrogate neutralizing activities were quantified by Cy5-labeld ACE2. The representative images from no antibody, low anti-S, and high anti-S were listed. **b** A dose dependent detection of anti-S bindings and ACE2 bindings on the wild-type SARS-CoV-2 spike protein. **c** Quantification of anti-S signals and normalized with anti-6x His signals. **d** Quantification of neutralization was calculated by the surrogate virus neutralization was indicated by the inhibition of ACE2 binding = 1 – (ACE2 with antibody/ACE2 without antibody) x 100%. **e** Quantification of anti-S signals across wild-type and variants. **f** Quantification of surrogate neutralize activities across wild-type and variants. Regression curve, r square, and IC_{50} were calculated with sigmoidal 4 pl model.

proof of concept, a humanized anti-S antibody was used to simultaneously detect the specificity of the antibody and ACE2 bindings against wild-type and common variants. Anti-S bound to wild-type spike protein and dose-dependently inhibited the ACE2 bindings (Fig. 2b). The surrogate virus neutralization was indicated by the inhibition of ACE2 binding = 1- (ACE2 binding with antibody/ACE2 binding without antibody) x 100%. Based on the anti-S dose-response curve, the top, bottom, and IC50 were 101%, 4%, and 767 pg, respectively (Fig. 2c). The ACE2 inhibition curve also showed a similar pattern, the top, the bottom, and the IC50 were 95%, 5%, and 1516 pg, respectively (Fig. 2d). Interestingly, the humanized anti-S showed broad bindings and surrogate neutralizing activities across the wild-type and eight common variants (Fig. 2e and f). This assay platform could accelerate the research and development of antibody drugs and other therapeutics.

An ELISA-based neutralization detection kit produced by GenScript cPass was recently received emergency use authorization from the Food and Drug Administration. Given the cPass ELISA kit only measured the neutralizing antibody for wild-type RBD, we selected the wild-type data on the CoVariant array for comparison. By using the WHO reference panel with high to low reactivities, we identified a high correlation of surrogate neutralizing activity between CoVariant and cPass methods with 0.973 r square value (Fig. S1a). We also showed the high correlation of surrogate neutralizing activity between CoVariant and reference values with 0.983 r square value (Fig. S1b). Moreover, serum IgG level measured by CoVariant array was highly correlated with reference values with 0.977–0.984 r square value (Figs. S1c and S1d). Thus, we believed the CoVariant arrays and assays are not only high throughput but also comparable with other measurements or units.

2.3. *Surrogate neutralizing activities in partial and fully vaccinated subjects*

To survey the immune response after vaccination, we collected sera from unvaccinated subjects (UN), individuals who received either one

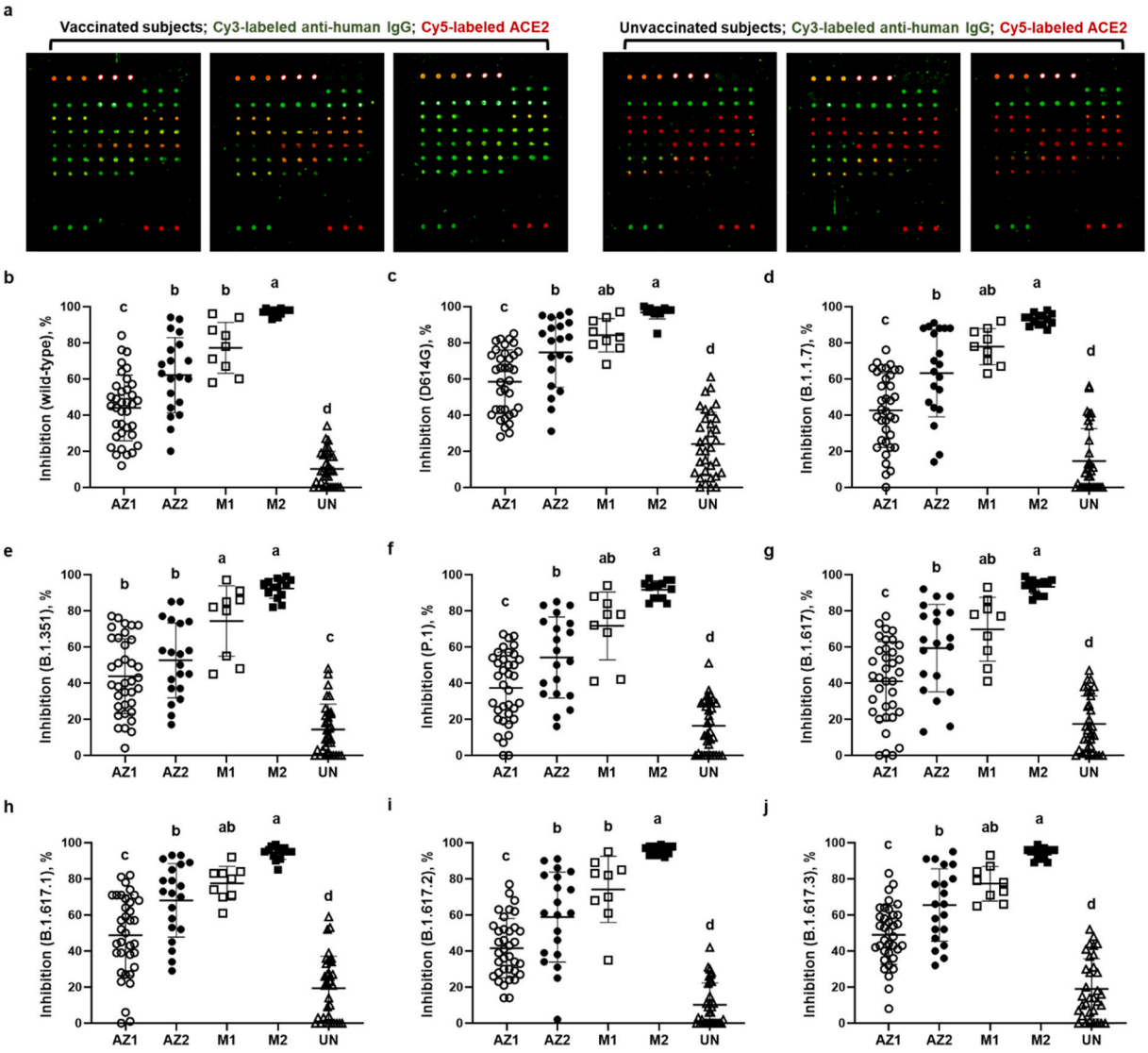

Fig. 3. High throughput detection of surrogate neutralizing activities against wild-type and common SARS-CoV-2 variants in partial and fully vaccinated subjects. Sera from partial, fully, or unvaccinated subjects were collected and analyzed for their surrogate neutralize activities by using CoVariant protein arrays. **a** Serum profiling images from 3 fully vaccinated and 3 unvaccinated subjects. The Cy3 green channel indicated the IgG amounts. The Cy5 red channel indicated the ACE2 bindings. **b** Surrogate neutralizing activities against wild-type SARS-CoV-2 in partial, fully, and unvaccinated subjects. **c-j** Surrogate neutralizing activities against eight common SARS-CoV-2 variants. AZ, M, and UN referred to AZD1222, mRNA-1273, and unvaccinated respectively. AZ1 n = 36, AZ2 n = 20, M1 n = 9, M2 n = 14, and UN n = 33. Data were analyzed by one-way ANOVA followed by Tukey's post-hoc tests, $p < 0.05$ between different letters. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

dose of AZD1222 (AZ1), two doses of AZD1222 (AZ2), or one dose of mRNA-1273, (M1), or two doses of mRNA-1273 (M2). The mean and standard deviation of the days after vaccination were 58.7 ± 9.0, 59.1 ± 24.9, 63.9 ± 28.6, 57.1 ± 28.9 for AZ1, AZ2, M1, and M2, respectively. Collected sera were used for high throughput profiling for their antibody specificities and surrogate neutralizing activities (Fig. 3a).

The surrogate neutralizing antibodies against wild-type and eight variant spike proteins were significantly higher after one or two doses of vaccines (Fig. 3b-j and Fig. S2). In wild-type and B.1.617.2 spike proteins, the surrogate neutralizing antibodies were the highest in M2, followed by M1 or AZ2, followed by AZ1, and the lowest in UN (Fig. 3b and i). In D614G, B.1.1.7, P.1, B.1.617, B.1.617.1, and B.1.617.3 spike variants, the surrogate neutralizing activities were M2 ≥ M1 ≥ AZ2 > AZ1 > UN (Fig. 3c, d, 3f-h, 3j). In B.1.351 spike protein, the surrogate neutralizing activity was M2 = M1 > AZ2 = AZ1 > UN (Fig. 3e). Although the surrogate neutralizing activities among vaccines were slightly different in wild-type and eight variants, they all share the similar tendency of higher in two doses than one dose and higher in mRNA-1273 than AZD1222. After being fully vaccinated, the M2 showed greater surrogate neutralizing activities than the AZ2 against all the wild-type and eight variants (Fig. 3 and Fig. S2).

2.4. IgG/A/M bindings in partial and fully vaccinated subjects

IgG is one of the most important immune responses after COVID-19 vaccination (Wei et al., 2021). To evaluate the COVID-19 vaccines against multiple variants, we quantified the IgG signals in AZ1, AZ2, M1, M2, and UN. The IgG levels against wild-type and variants were higher in either one or two doses of vaccines, except for B.1.617 (Fig. 4). In AZD1222, both partial or fully vaccinated subjects generated a significant amount of IgG against all the wild-type and eight variants. However, in AZD1222, two doses did not induce more IgG than one dose (Fig. 4). In contrast, M2 evoked more IgG than M1 in most spike variants (Fig. 4 and Fig. S3). This may partially explain the better surrogate neutralizing activities in M2 than AZ2.

IgA is the most prevalent isotype within the mucosal membranes and plays an important role in mucosal immunity which might help to fight against invading SARS-CoV-2. Only a few studies addressed the IgA levels after natural infection or vaccination (Du et al., 2021; Jalkanen et al., 2021). Here, we evaluated the IgA responses against multiple variants in partial and fully vaccinated subjects. In mRNA-1273, neither one dose nor two doses did not induce IgA (Fig. 5 and Fig. S4). In contrast, the two doses of AZD1222 evoked a significant amount of IgA against wild-type and variants, except for B.1.617 and B.1.617.2 (Fig. 5 and Fig. S4). The increment of IgA after two doses of AZD1222 may contribute in part to the surrogate neutralizing activities.

IgM plays a major role in the surrogate neutralizing activities in COVID-19 convalescent patients (Gasser et al., 2021). We quantified the IgM responses against multiple variants in partial and fully vaccinated subjects. In AZD1222, neither one nor two doses did not induce IgM (Fig. S5 and Fig. S6). Interestingly, two doses of mRNA-1273 significantly increased the IgM level against wild-type and variants, except for B.1.617 and B.1.617.2 (Fig. S5 and Fig. S6). The elevation of IgG and IgM after two doses of mRNA-1273 may in part contribute to the surrogate neutralizing activities.

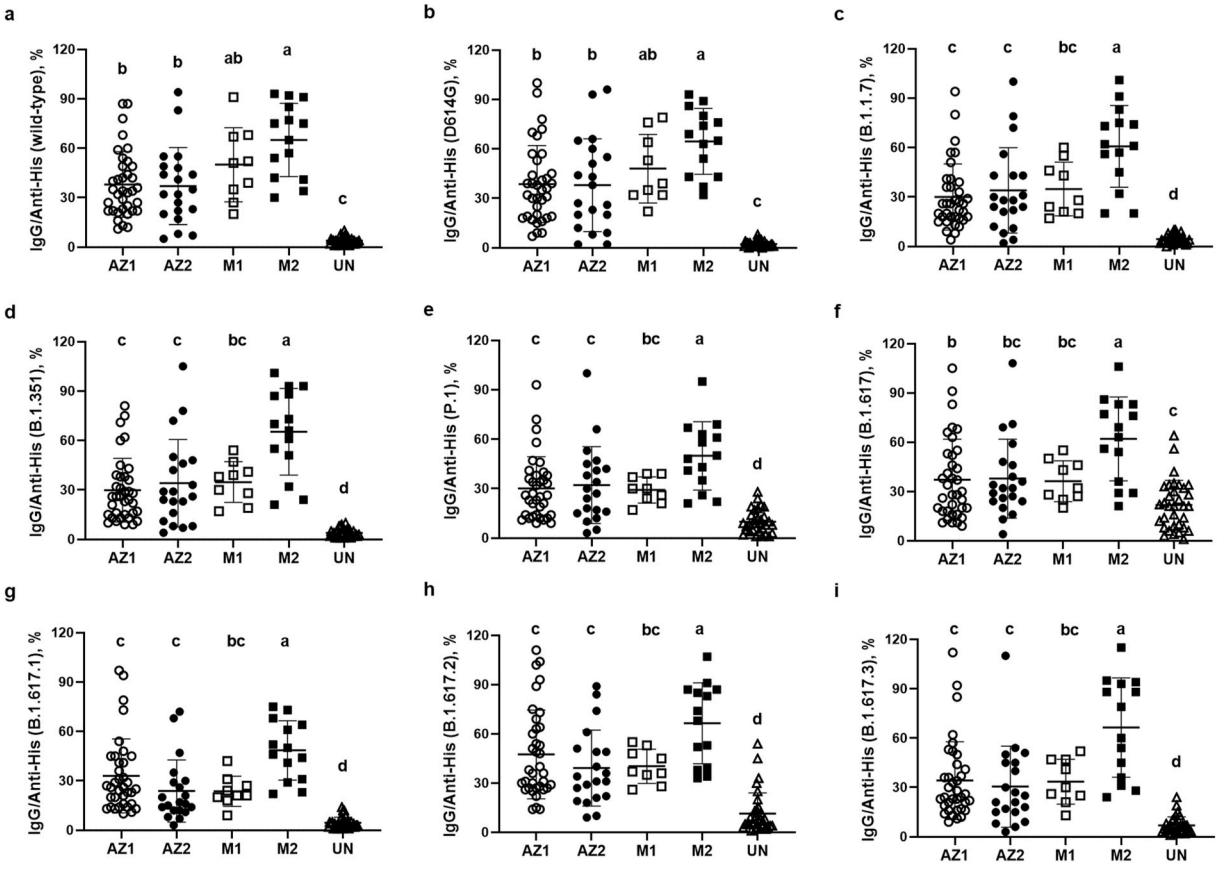

Fig. 4. High throughput detection of serum IgG against wild-type and common SARS-CoV-2 variants in partial and fully vaccinated subjects.
Sera from partial, fully, or unvaccinated subjects were collected and analyzed for their IgG bindings by using CoVariant protein arrays. **a** IgG bindings against wild-type SARS-CoV-2 in partial, fully, and unvaccinated subjects. **c-i** IgG bindings against eight common SARS-CoV-2 variants. Data were analyzed by one-way ANOVA followed by Tukey's post-hoc tests, $p < 0.05$ between different letters.

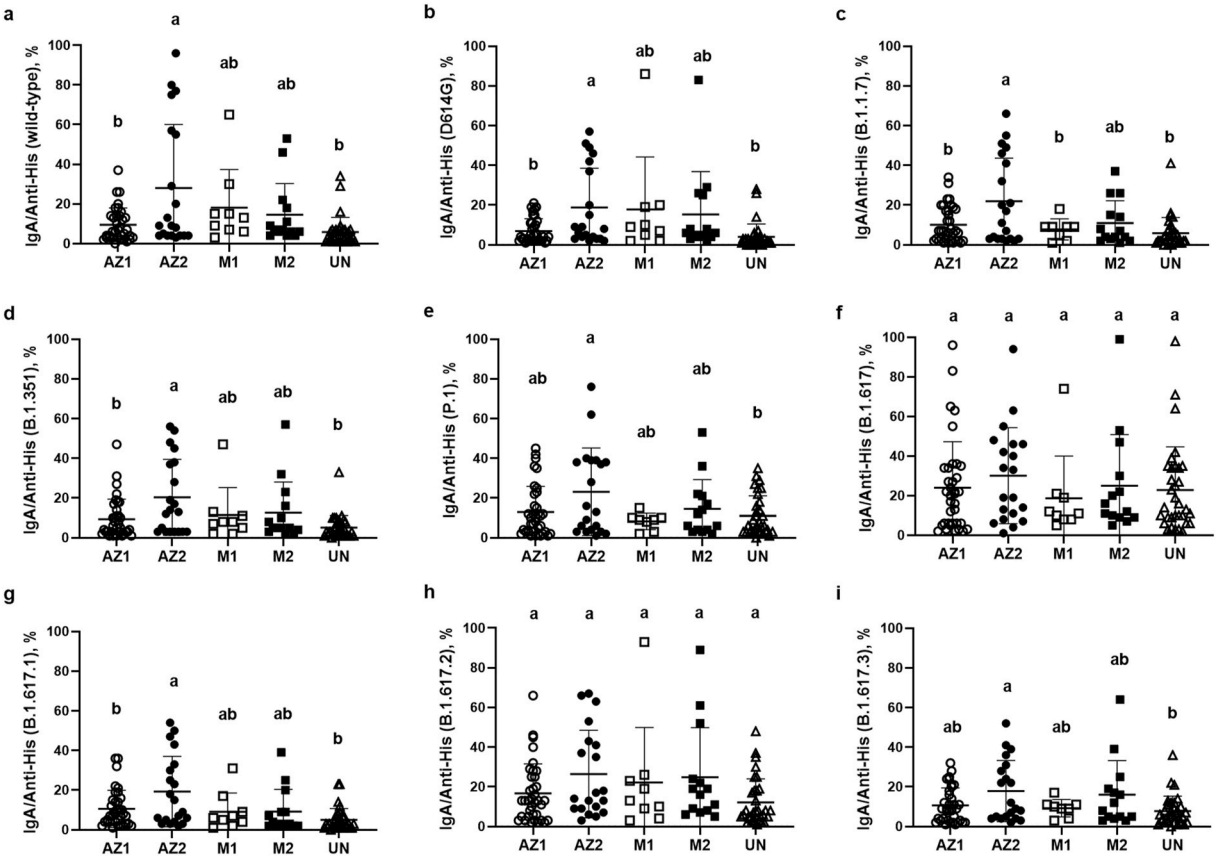

Fig. 5. High throughput detection of serum IgA against wild-type and common SARS-CoV-2 variants in partial and fully vaccinated subjects.
Sera from partial, fully, or unvaccinated subjects were collected and analyzed for their IgA bindings by using CoVariant protein arrays. **a** IgA bindings against wild-type SARS-CoV-2 in partial, fully, and unvaccinated subjects. **c-i** IgA bindings against eight common SARS-CoV-2 variants. Data were analyzed by one-way ANOVA followed by Tukey's post-hoc tests, $p < 0.05$ between different letters.

2.5. Crosstalk between antibody isotypes and surrogate neutralizing activities

Identifying the antibody response to SARS-CoV-2 and possible protection is crucial for global health security and the development of more effective vaccines. To analyze the relationship between antibody isotypes and the surrogate neutralizing activities, we performed global correlation analysis with all the subjects against wild-type spike protein (Figs. S7a–c). IgG, IgA, and IgM levels were significantly correlated with surrogate neutralizing activities. If focusing on AZD1222, the IgM was not related to surrogate neutralizing activities (Figs. S7d–f). If focusing on mRNA-1273, all three isotypes were significantly correlated with surrogate neutralizing activities (Figs. S7g–i). While the IgG level increased in AZ1, AZ2, M1, and M2, the IgA and IgM level only evoked in AZ2 and M2, respectively (Fig. S8). This indicated a distinct humoral immune regulation between vector and mRNA vaccines.

3. Discussion

Reduced morbidity and death in the elderly and other vulnerable populations is a significant priority for mass vaccination efforts, particularly in the context of limited vaccine supply and VOC onset. All of the SARS-CoV-2 variants differ in two critical regions of the spike protein that are recognized by our body's immune system's neutralizing antibodies. These regions are the RBD which can interact with the ACE2 receptor (Liu et al., 2020; Zhang et al., 2021) and the N-terminal domain (NTD) which is a prime target for B cells after vaccination (Carreño et al., 2021). Mutations in some of these regions can reduce the neutralizing antibodies to attach to the spike. In this study, we developed a multiplexed CoVariant protein microarray by immobilizing the wild-type and eight spike ECD variants on a chip. Since the ECD contained both RBD and NTD, we were able to determine the amount of antibody and neutralization on each spike variant in a single experiment. In addition, if there is a new VOC such as omicron in the future, we will express that spike ECD and expand it on the CoVariant protein array.

While IgG, IgA, and IgM isotypes were found to be associated with surrogate neutralizing activities, they behaved differently after vaccination. In AZD1222, the IgG level was increased after one dose and remained at a high level after the second dose. Only after two doses of AZD1222 were able to induce the IgA level. As a result, the surrogate neutralizing activity was higher in two doses than one dose of AZD1222. In mRNA-1273, the IgG level was elevated after one dose and further evoked after the second dose. The IgM level was also evoked after the second dose of mRNA-1273. Moreover, the IgM level played a key role in the ability of the convalescent plasma to neutralize SARS-CoV-2 (Gasser et al., 2021). As a result, the two doses of mRNA-1273 showed better surrogate neutralizing activity than the two doses of AZD1222. Although the detailed mechanisms for the isotype differences in AZD1222 and mRNA-1273 were not clear, it agreed with recent findings that suggest the mixing of these two vaccines generates stronger humoral responses (Schmidt et al., 2021).

Humoral responses take the form of numerous antibody subtypes, among which IgG is the most abundant and long-lived (Wisnewski et al., 2021). We found the abundant elevation of IgG isotypes against the spike and its variants after partially and fully vaccination may indicate long-term protection. Our findings were consistent with others that showed dominant IgG isotypes in either vector or mRNA vaccines (Ewer

et al., 2021; Jalkanen et al., 2021). Among humoral responses, the surrogate neutralizing activities were the most valuable parameters for mimicking the infection. Several studies have shown a link between the level of neutralization and the risk of infection for SARS-CoV-2 (Addetia et al., 2020; Khoury et al., 2021a). As a result, the quantity of the humoral response after immunization, which coincides with neutralizing antibody titers (Khoury et al., 2021b), may be clinically significant.

4. Conclusion

In this study, we developed CoVariant arrays and surrogate neutralizing assays to facilitate the biochemical studies and immune assessments of the SARS-CoV-2 variants. The results showed a broad binding spectrum and surrogate neutralizing activities of the antibody drugs toward multiple variants. By using CoVariant arrays and assays, we investigated the humoral immune responses against multiple variants after partial or full vaccination with either AZD1222 or mRNA-1273. The future directions would be utilizing the CoVariant protein array to evaluate cellular immune responses after vaccinations as well as integrating the Omicron variant into the array to further evaluate the existing vaccines.

5. Methods

5.1. Fabrication of CoVariant protein microarray

For the fabrication of multiplexed SARS-CoV-2 Variant (CoVariant) protein microarrays, twenty-three viral proteins including the spike proteins, nucleocapsid proteins, 4 cell membranes, and 8 controls were mixed with 30% glycerol and transferred into the 384 well as source plate for printing (Table S1). The cell membrane was prepared by harvesting 5×10^6 cells, washed, and extracted by a membrane isolation kit (ThermoFisher, #89842). The slides were precoated with aldehyde and stored at 4 °C as previously described (Du et al., 2021). Each sample was printed in triplicate in each block (9 × 10 format) and 14 identical blocks per slide using a contact printer (CapitalBio, #SmartArrayer 136). After printing, the CoVariant protein microarrays were immobilized overnight, vacuum-sealed, and stored at 4 °C for a short term (<6 months) or at −80 °C for a long term (6 months to a few years). The immobilization and functionality of the proteins on the microarrays were assessed by anti-His, anti-spike, and ACE2.

5.2. Quality control of CoVariant protein microarray

The CoVariant protein microarrays stored at −80 °C were thawed to room temperature, washed with 1X TBST (TBS with 0.1% Tween 20) for 10 min, blocked by SuperBlock buffer (ThermoFisher, #37537) for 15 min, and incubated with 50 μL of 647-conjugated Rabbit Anti-His tag 1.1 ng/mL (Jackson Lab, #300-605-240), anti-spike antibody 31.25 ng/mL (Sino Biological, #40150-D001) with anti-human IgG conjugated with Cy3 1.5 μg/mL (Jackson Laboratory, #109-165-003), or biotinylated ACE2 125 pg/mL (Sino Biological, #10108-H08H–B) with Cy5-conjugated Streptavidin 2 ng/mL (Jackson Lab, #016-170-084) for quality control. After several washes, the arrays were dried and scanned then scanned for Cy3 and Cy5 signals with power 25% and 30% (Caduceus Biotechnology, #SpinScan).

5.3. Antibody and receptor specificity profiling

Chimeric monoclonal antibodies against the SARS-CoV-2 spike (Sino Biological, #40150-D001) were used to compete with ACE2 bindings. After blocking, the CoVariant array was incubated with 50 μL of serially diluted monoclonal antibodies for 1 h, and washed again with TBST. Followed by incubation with 50 μL of Cy5-conjugated Streptavidin 2 ng/mL (Jackson Laboratory, #016-170-084), biotinylated ACE2 125 pg/mL (Sino biological, #10108-H08H–B), and anti-human IgG conjugated with Cy3 1.5 μg/mL (Jackson Laboratory, #109-165-003) for 1 h, washed, dried, and then scanned.

5.4. Subjects and ethical statement

Blood samples were obtained with standard aseptic phlebotomy technique at least 20 days after the first dose of vaccination or at least 7 days after the second dose of vaccinations. Each participant took 10 mL of blood using sodium heparin BD vacutainer collection tubes (5 mL; Becton Drive Vacutainer, Franklin Lakes, USA). Serum was separated and stored at −80 °C until use. Participants who have available blood samples and signed informed consents were included. While those who have severe, chronic illnesses such as hepatomegaly, severe anemia, bleeding disorders, or any known pre-existing immunologic disorders, having received blood products 6 months prior to the day of enrollment were excluded. All participants gave their informed consent before they participated in the study. The study was conducted adhering to the Declaration of Helsinki, and the protocols were approved and conducted following the Institutional Review Boards of National Cheng Kung University Hospital (NCKUH) (reference number A-ER-110-203), which is organized and operated according to the laws and regulations of Good Clinical Practice (ICH-GCP).

5.5. Serum profiling

After blocking, the CoVariant array was incubated with 50 μL of 50-fold diluted serum in TBST supplemented with 1% BSA for an hour and washed. Followed by incubation with 50 μL of biotinylated human ACE2 125 pg/mL, and Cy5-conjugated streptavidin 2 ng/mL, Cy3-labeled anti-human IgG/A/M antibodies (Jackson Laboratory, 75 ng/ml #109-165-008, 150 ng/ml #109-165-011, and 1.5 μg/ml #109-165-043) for an hour. After final washes, the arrays were dried and scanned for Cy3 and Cy5 signals.

5.6. Data analysis

The signals were analyzed by GenePix Pro software as foreground minus background. To calculate the competition assay (surrogate neutralizing activity), data from the sample and buffer were both needed. The surrogate virus neutralization was indicated by the inhibition of ACE2 binding = 1 – (ACE2 with sample/ACE2 without sample) x 100%. For IgG/A/M quantification, the fluorescence signals from spike proteins were divided by their His signals to normalize the protein amounts. The dose-response curve was analyzed by the sigmoidal 4 pl model. The correlation was analyzed by the linear regression model. Comparison between multiple groups was done by one-way ANOVA followed by Tukey's post-hoc tests with a $p < 0.05$ as the threshold for significance. The outliers were identified by Grubbs' method with alpha 0.0001. All the statistics and figures were done by GraphPad Prism software. All data were presented as mean ± SD, where n was the number of subjects.

Data availability

The list of materials on the CoVariant array was attached in Supplementary Table 1. The datasets generated during and/or analyzed during the current study are available from the corresponding author upon request.

CRediT authorship contribution statement

Tzong-Shiann Ho: performed experimental work, contributed to manuscript preparation, contributed their expertise and supervision to the entire project. **Pin-Xian Du:** contributed to manuscript preparation. **Wen-Yu Su:** performed experimental work, contributed to manuscript preparation. **Harvey M. Santos:** contributed to manuscript preparation,

Writing – original draft. **Ya-Lan Lin:** performed experimental work. **Yi-Yu Chou:** performed experimental work. **Batuhan Birol Keskin:** performed experimental work. **Chi Ho Pau:** performed experimental work. **Guan-Da Syu:** performed experimental work, contributed to manuscript preparation, contributed their expertise and supervision to the entire project, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2022.114067.

References

Addetia, A., Crawford, K.H.D., Dingens, A., Zhu, H., Roychoudhury, P., Huang, M.L., Jerome, K.R., Bloom, J.D., Greninger, A.L., 2020. Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a fishery vessel outbreak with a high attack rate. J. Clin. Microbiol. 58 (11).

Carreño, J.M., Alshammary, H., Tcheou, J., Singh, G., Raskin, A., Kawabata, H., Sominsky, L., Clark, J., Adelsberg, D.C., Bielak, D., Gonzalez-Reiche, A.S., Dambrauskas, N., Vigdorovich, V., Alburquerque, B., Amoako, A., Banu, R., Beach, K., Bermúdez-González, M.C., Cai, G., Ceglia, I., Cognigni, C., Farrugia, K., Gleason, C., van de Guchte, A., Kleiner, G., Khalil, Z., Lyttle, N., Mendez, W., Mulder, L.C.F., Oostenink, A., Rooker, A., Salimbangon, A., Saksena, M., Paniz-Mondolfi, A., Polanco, J., Srivastava, K., Sather, D.N., Sordillo, E.M., Bajic, G., van Bakel, H., Simon, V., Krammer, F., Group, P.P.S., 2021. Activity of convalescent and vaccine serum against SARS-CoV-2 Omicron. Nature.

de Assis, R.R., Jain, A., Nakajima, R., Jasinskas, A., Felgner, J., Obiero, J.M., Norris, P.J., Stone, M., Simmons, G., Bagri, A., Irsch, J., Schreiber, M., Buser, A., Holbro, A., Battegay, M., Hosimer, P., Noesen, C., Adenaiye, O., Tai, S., Hong, F., Milton, D.K., Davies, D.H., Contestable, P., Corash, L.M., Busch, M.P., Felgner, P.L., Khan, S., 2021. Analysis of SARS-CoV-2 antibodies in COVID-19 convalescent blood using a coronavirus antigen microarray. Nat. Commun. 12 (1), 6.

Du, P.-X., Chou, Y.-Y., Santos, H.M., Keskin, B.B., Hsieh, M.-H., Ho, T.-S., Wang, J.-Y., Lin, Y.-L., Syu, G.-D., 2021. Development and application of human coronavirus protein microarray for specificity analysis. Anal. Chem. 93 (21), 7690–7698.

Emary, K.R., Golubchik, T., Aley, P.K., Ariani, C.V., Angus, B., Bibi, S., Blane, B., Bonsall, D., Cicconi, P., Charlton, S., 2021. Efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 variant of concern 202012/01 (B. 1.1. 7): an exploratory analysis of a randomised controlled trial. Lancet 397 (10282), 1351–1362.

Ewer, K.J., Barrett, J.R., Belij-Rammerstorfer, S., Sharpe, H., Makinson, R., Morter, R., Flaxman, A., Wright, D., Bellamy, D., Bittaye, M., Dold, C., Provine, N.M., Aboagye, J., Fowler, J., Silk, S.E., Alderson, J., Aley, P.K., Angus, B., Berrie, E., Bibi, S., Cicconi, P., Clutterbuck, E.A., Chelysheva, I., Folegatti, P.M., Fuskova, M., Green, C.M., Jenkin, D., Kerridge, S., Lawrie, A., Minassian, A.M., Moore, M., Mujadidi, Y., Plested, E., Poulton, I., Ramasamy, M.N., Robinson, H., Song, R., Snape, M.D., Tarrant, R., Voysey, M., Watson, M.E.E., Douglas, A.D., Hill, A.V.S., Gilbert, S.C., Pollard, A.J., Lambe, T., Oxford, C.V.T.G., 2021. T cell and antibody responses induced by a single dose of ChAdOx1 nCoV-19 (AZD1222) vaccine in a phase 1/2 clinical trial. Nat. Med. 27 (2), 270–278.

Gasser, R., Cloutier, M., Prevost, J., Fink, C., Ducas, E., Ding, S., Dussault, N., Landry, P., Tremblay, T., Laforce-Lavoie, A., Lewin, A., Beaudoin-Bussieres, G., Laumaea, A., Medjahed, H., Larochelle, C., Richard, J., Dekaban, G.A., Dikeakos, J.D., Bazin, R., Finzi, A., 2021. Major role of IgM in the neutralizing activity of convalescent plasma against SARS-CoV-2. Cell Rep. 34 (9), 108790.

Hall, V.J., Foulkes, S., Saei, A., Andrews, N., Oguti, B., Charlett, A., Wellington, E., Stowe, J., Gillson, N., Atti, A., 2021. COVID-19 vaccine coverage in health-care workers in England and effectiveness of BNT162b2 mRNA vaccine against infection (SIREN): a prospective, multicentre, cohort study. Lancet 397 (10286), 1725–1735.

Jalkanen, P., Kolehmainen, P., Hakkinen, H.K., Huttunen, M., Tahtinen, P.A., Lundberg, R., Maljanen, S., Reinholm, A., Tauriainen, S., Pakkanen, S.H., Levonen, I., Nousiainen, A., Miller, T., Valimaa, H., Ivaska, L., Pasternack, A., Naves, R., Ritvos, O., Osterlund, P., Kuivanen, S., Smura, T., Hepojoki, J., Vapalahti, O., Lempainen, J., Kakkola, L., Kantele, A., Julkunen, I., 2021. COVID-19 mRNA vaccine induced antibody responses against three SARS-CoV-2 variants. Nat. Commun. 12 (1), 3991.

Jiang, H.-w., Li, Y., Zhang, H.-n., Wang, W., Yang, X., Qi, H., Li, H., Men, D., Zhou, J., Tao, S.-c., 2020. SARS-CoV-2 proteome microarray for global profiling of COVID-19 specific IgG and IgM responses. Nat. Commun. 11 (1), 3581.

Khoury, D.S., Cromer, D., Reynaldi, A., Schlub, T.E., Wheatley, A.K., Juno, J.A., Subbarao, K., Kent, S.J., Triccas, J.A., Davenport, M.P., 2021a. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat. Med. 27 (7), 1205–1211.

Khoury, D.S., Cromer, D., Reynaldi, A., Schlub, T.E., Wheatley, A.K., Juno, J.A., Subbarao, K., Kent, S.J., Triccas, J.A., Davenport, M.P., 2021b. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat. Med. 27 (7), 1205–1211.

Liu, L., Wang, P., Nair, M.S., Yu, J., Rapp, M., Wang, Q., Luo, Y., Chan, J.F., Sahi, V., Figueroa, A., Guo, X.V., Cerutti, G., Bimela, J., Gorman, J., Zhou, T., Chen, Z., Yuen, K.Y., Kwong, P.D., Sodroski, J.G., Yin, M.T., Sheng, Z., Huang, Y., Shapiro, L., Ho, D.D., 2020. Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike. Nature 584 (7821), 450–456.

Ma, M.L., Shi, D.W., Li, Y., Hong, W., Lai, D.Y., Xue, J.B., Jiang, H.W., Zhang, H.N., Qi, H., Meng, Q.F., Guo, S.J., Xia, D.J., Hu, J.J., Liu, S., Li, H.Y., Zhou, J., Wang, W., Yang, X., Fan, X.L., Lei, Q., Chen, W.J., Li, C.S., Yang, X.M., Xu, S.H., Wei, H.P., Tao, S.C., 2021. Systematic profiling of SARS-CoV-2-specific IgG responses elicited by an inactivated virus vaccine identifies peptides and proteins for predicting vaccination efficacy. Cell Discov. 7 (1), 67.

Schmidt, T., Klemis, V., Schub, D., Mihm, J., Hielscher, F., Marx, S., Abu-Omar, A., Ziegler, L., Guckelmus, C., Urschel, R., Schneitler, S., Becker, S.L., Gartner, B.C., Sester, U., Sester, M., 2021. Immunogenicity and reactogenicity of heterologous ChAdOx1 nCoV-19/mRNA vaccination. Nat. Med. 27 (9), 1530–1535.

Syu, G.-D., Dunn, J., Zhu, H., 2020. Developments and applications of functional protein microarrays. Mol. Cell. Proteomics 19 (6), 916–927.

Tan, C.W., Chia, W.N., Qin, X., Liu, P., Chen, M.I., Tiu, C., Hu, Z., Chen, V.C., Young, B.E., Sia, W.R., Tan, Y.J., Foo, R., Yi, Y., Lye, D.C., Anderson, D.E., Wang, L.F., 2020. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction. Nat. Biotechnol. 38 (9), 1073–1078.

Wei, J., Stoesser, N., Matthews, P.C., Ayoubkhani, D., Studley, R., Bell, I., Bell, J.I., Newton, J.N., Farrar, J., Diamond, I., Rourke, E., Howarth, A., Marsden, B.D., Hoosdally, S., Jones, E.Y., Stuart, D.I., Crook, D.W., Peto, T.E.A., Pouwels, K.B., Eyre, D.W., Walker, A.S., team, C.-I.S., 2021. Antibody responses to SARS-CoV-2 vaccines in 45,965 adults from the general population of the United Kingdom. Nat. Microbiol. 6 (9), 1140–1149.

Wisnewski, A.V., Campillo Luna, J., Redlich, C.A., 2021. Human IgG and IgA responses to COVID-19 mRNA vaccines. PLoS One 16 (6), e0249499.

Zhang, J., Xiao, T., Cai, Y., Lavine, C.L., Peng, H., Zhu, H., Anand, K., Tong, P., Gautam, A., Mayer, M.L., Walsh Jr., R.M., Rits-Volloch, S., Wesemann, D.R., Yang, W., Seaman, M.S., Lu, J., Chen, B., 2021. Membrane fusion and immune evasion by the spike protein of SARS-CoV-2 Delta variant. Science, eabl9463.