Received: 8 November 2023 | Accepted: 14 April 2024

DOI: 10.1002/jmv.29628

RESEARCH ARTICLE

Antibody-dependent enhancement (ADE) of SARS-CoV-2 in patients exposed to MERS-CoV and SARS-CoV-2 antigens

Swapna Thomas[1,2] | Maria K. Smatti[1] | Haya Alsulaiti[1,3] | Hadeel T. Zedan[1,4] | Ali H. Eid[5] | Ali A. Hssain[6] | Laith J. Abu Raddad[7] | Giusy Gentilcore[8] | Allal Ouhtit[2] | Asmaa A. Althani[1,3] | Gheyath K. Nasrallah[1,4] | Jean-Charles Grivel[8] | Hadi M. Yassine[1,4]

[1]Biomedical Research Center, Research Complex, Qatar University, Doha, Qatar

[2]Department of Biological and Environmental Sciences, College of Arts and Sciences, Qatar University, Doha, Qatar

[3]QU Health, Qatar University, Doha, Qatar

[4]Department of Biomedical Science, College of Health Sciences-QU Health, Qatar University, Doha, Qatar

[5]College of Medicine-QU Health, Qatar University, Doha, Qatar

[6]Medical Intensive Care Unit, Hamad Medical Corporation, Doha, Qatar

[7]Infectious Disease Epidemiology Group, Department of Population Health Sciences, Weill Cornell Medicine-Qatar, Doha, Qatar

[8]Deep Phenotyping Core, Sidra Medicine, Doha, Qatar

Correspondence
Hadi M. Yassine Ph.D., Biomedical Research Center, Research Complex, Qatar University, P.O. Box 2713, Doha, Qatar.
Email: hyassine@qu.edu.qa

Funding information
This study was supported by partial funds from QU., Grant/Award Number: QUCG-BRC-2022/23-578

Abstract

This study evaluated the potential for antibody-dependent enhancement (ADE) in serum samples from patients exposed to Middle East respiratory syndrome coronavirus (MERS-CoV). Furthermore, we evaluated the effect of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination on ADE in individuals with a MERS infection history. We performed ADE assay in sera from MERS recovered and SARS-CoV-2-vaccinated individuals using BHK cells expressing FcgRIIa, SARS-CoV-2, and MERS-CoV pseudoviruses (PVs). Further, we analyzed the association of ADE to serum IgG levels and neutralization. Out of 16 MERS patients, nine demonstrated ADE against SARS-CoV-2 PV, however, none of the samples demonstrated ADE against MERS-CoV PV. Furthermore, out of the seven patients exposed to SARS-CoV-2 vaccination after MERS-CoV infection, only one patient (acutely infected with MERS-CoV) showed ADE for SARS-CoV-2 PV. Further analysis indicated that IgG1, IgG2, and IgG3 against SARS-CoV-2 S1 and RBD subunits, IgG1 and IgG2 against the MERS-CoV S1 subunit, and serum neutralizing activity were low in ADE-positive samples. In summary, samples from MERS-CoV-infected patients exhibited ADE against SARS-CoV-2 and was significantly associated with low levels of neutralizing antibodies. Subsequent exposure to SARS-CoV-2 vaccination resulted in diminished ADE activity while the PV neutralization assay demonstrated a broadly reactive antibody response in some patient samples.

1 | INTRODUCTION

Antibody-dependent enhancement (ADE) is an alternative mechanism of infection used by many viruses including respiratory viruses. Antibodies at subneutralizing concentrations are advantageous to certain viruses because they facilitate viral entry into cells expressing antibody receptors, including Fc gamma receptors (FcgR) or complement receptors (CR).[1–3] Both FcgR and CR-mediated ADE are reported in SARS-CoV-2 infection.[4] Hawkes et al. first reported this phenomenon in 1964 in arboviruses[5] and later by many studies on

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dengue virus.[6–8] Later, ADE was reported in respiratory viruses, specifically in infections following vaccinations with RSV,[9,10] influenza,[3,11] or measles (inactivated virus).[12] Further ADE was reported in vitro and in animal models for coronaviruses, including severe acute respiratory syndrome coronavirus (SARS-CoV)[13] and Middle East respiratory syndrome coronavirus (MERS-CoV).[14] With the emergence of the COVID-19 pandemic in 2019,[15] ADE after coronavirus infection has been actively investigated. The betacoronavirus SARS-CoV-2 has ~80% sequence similarity with SARS-CoV and ~54% sequence similarity with MERS-CoV.[16,17] This similarity supports the hypothesis that ADE may be possible after SARS-CoV-2 infection in people with previous infection by SARS-CoV or MERS-CoV. ADE after SARS-CoV-2 infection, including severe infection, has been reported in in vitro studies at sub-neutralizing antibody concentrations.[4,18–20] Antibodies that induce ADE have been reported to persist for as many as 6 months in patients who have recovered from COVID-19.[21]

ADE occurs mainly via the FcgR (antibody receptors), including FcgRIIa, FcgRIIb, and FcgRIIIa, which are expressed in immune cells.[12,22,23] The ligand for FcgRIIa and FcgRIIb receptors is on the Fc region of IgG.[12,24] FcgRIIa and FcgRIIb are low-affinity IgG receptors that engage with high affinity virus-complexed with several virus-specific antibodies, including influenza, HIV, Ebolavirus, and coronaviruses.[2,21,25] In vitro studies demonstrated that antibodies at lower concentrations could exacerbate enhanced virus entry (ADE) into FcgR-expressing cells ADE.[26–28]

Cross-reactivity between SARS-CoV-2 and other coronaviruses, including SARS-CoV and MERS-CoV, has been reported.[29–31] It is possible that if such antibodies are present at sub-neutralizing concentration, ADE may arise after infection with other coronaviruses including new variants of SARS-CoV-2. So far, 2604 confirmed MERS cases, including 936 deaths, have been reported globally (until February 2023).[32] MERS was first reported in Saudi Arabia in 2012 and has spread to 27 countries. Qatar reported 28 human cases, including two cases in March 2022, and seven deaths until April 2022.[33] Pre-existing immunity against SARS and MERS is a major concern regarding the potential for ADE after the emergence of SARS-CoV-2 variants. Here, we investigated ADE in samples of patients previously infected with MERS-CoV and examined how SARS-CoV-2 vaccination might shape ADE in these patients. Furthermore, we characterized the IgG subclasses and neutralization activity in these samples, in correlation with ADE.

2 | METHODS

2.1 | Patients and clinical samples

A total of 36 patients exposed to MERS-CoV (16 patients) and SARS-CoV-2 (20 patients) through infection/vaccination were included in this study (Table 1). Among 16 MERS patients, 14 had a history of MERS infection while 2 were too acutely infected. Seven MERS patients were vaccinated with the SARS-CoV-2 mRNA vaccine (BNT162b2 or mRNA-1273), four of them had serial samples before and after vaccination, and one with an acute MERS infection (previously vaccinated against COVID-19). Among 20 COVID-19 acute patients, 10 patients had severe infections and 10 had mild infections. All participants were men between 28 and 85 years of age —median age = 46 years. Since all MERS-infected patients were male, and we only included male COVID-19 patients for comparison. Eight samples from two acute MERS-infected ICU-admitted patients were collected at different days postinfection (DPI): Six and two samples were collected from the first (survived) and the second patient (deceased), respectively (Table S1). We randomly selected six age- and gender-matched control samples (prepandemic) for the analysis (Table S2).

2.2 | Generation and titration of pseudoviruses

Pseudoviruses (PVs) expressing S proteins of wild-type SARS-CoV-2 and MERS-CoV were produced in HEK293T (human embryonic kidney cells) using the recombinant ΔG-vesicular stomatitis virus (VSV ΔG) with a luciferase reporter as previously described.[34] The titration of produced PVs was done in HEK293 T cells expressing ACE2 receptor as described elsewhere.[35] The viral stock concentration was measured as luminescence with a TECAN infinite F200 PRO plate reader and calculated as luminescence signal (RLU) per well and then converted to RLU/mL.

2.3 | Characterization of FcgR-expressing cells

The BHK cells expressing FcgRIIa were generously provided by Yoshihiro Kawaoka et al.[21] To characterize the surface expression of FcgRIIa on BHK cells with immunofluorescence assay, we seeded BHK cells with/without FcgRIIa expression in six-well plates on a sterile coverslip. After overnight incubation at 37°C, the cells were fixed with 4% formaldehyde for 10 min, permeabilized with 1% Triton X-100 for 30 min, and blocked with 0.1% BSA for 60 min. The cells were then treated with FcgRIIa primary antibody (Invitrogen; Cat #PA5-102409) overnight and then with fluorescently labeled secondary antibody (Cat #A11029). The cells were subsequently stained with DAPI in 0.1% BSA and incubated for 10 min, then observed under a fluorescence microscope and images were captured.

2.4 | ADE assays

ADE assays were performed as previously described[36] with minor modifications. Heat-inactivated serum samples (30 min at 56°C) were serially diluted (fourfold) in DMEM high-glucose medium without FBS. The volume was adjusted to 100 µL and divided into two parts (duplicates). Then, 50 µL PVs (SARS-CoV-2 or MERS-CoV) at a concentration of 1×10^6 RLU/mL were added to each well and

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TABLE 1 Demographic and clinical characteristics of the patients.

Patient/sample ID	Gender	Age at the time of sample collection (years)		Date of sample collection	Days between Infection and sample collection
MERS convalescent samples					
MERS-1.i	M	28		Jan 29, 2021	18 months
MERS-2.i	M	34		Dec 4, 2021	20 months
MERS-3.i[a]	M	70		Dec 4, 2021	18 months
MERS-4.i	M	31		Dec 4, 2021	14 months
MERS-5.i	M	34		Oct 2016	8 months
MERS-6.i	M	35		Oct 2016	4 months
MERS-7.i	M	51		Oct 2016	2 months
MERS-8.i	M	41		Jul 2016	16 months
MERS-9.i	M	35		Jul 2016	15 months
MERS-10.i	M	51		May 2016	12 months
MERS-11.i	M	51		May 2016	12 months
MERS-12.i	M	34		May 2016	19 months
MERS convalescent samples with SARS-CoV-2 vaccination samples			Date of second dose adminis-tration		Days between vaccination and sample collection
MERS-1.i + SARS-CoV-2v	M	28	Jun 24, 2021	Oct 21, 2021	119
MERS-2.i + SARS-CoV-2v	M	34	Sep 3, 2021	Oct 20, 2021	47
MERS-3.i + SARS-CoV-2v	M	70	Apr 14, 2021	Oct 20, 2021	189
MERS-4.i + SARS-CoV-2v	M	31	Sep 11, 2021	Oct 20, 2021	39
MERS-13.i + SARS-CoV-2v	M	66	Mar 2, 2021	Aug 21, 2021	172
MERS-14.i + SARS-CoV-2v	M	54	Mar 16, 2021	Oct 24, 2021	222
Acute MERS samples					
MERS-15.ai	M	50	Non-vaccinated	Mar 21, 2022	
MERS-16.ai + SARS-CoV-2v	M	85	Mar 2, 2021	Mar 31, 2022	393
SARS-CoV-2 severe (infection) samples					
SARS-1.si	M	46	Non-vaccinated	Apr 27, 2020	
SARS-2.si	M	48	Non-vaccinated	Apr 27, 2020	
SARS-3.si	M	52	Non-vaccinated	Apr 27, 2020	
SARS-4.si	M	63	Non-vaccinated	Apr 28, 2020	
SARS-5.si	M	68	Non-vaccinated	Apr 28, 2020	
SARS-6.si	M	65	Non-vaccinated	Apr 28, 2020	
SARS-7.si	M	59	Non-vaccinated	Apr 28, 2020	
SARS-8.si	M	44	Non-vaccinated	Apr 28, 2020	
SARS-9.si	M	40	Non-vaccinated	Apr 29, 2020	
SARS-10.si	M	51	Non-vaccinated	Apr 29, 2020	
SARS-CoV-2 mild (infection) samples					
SARS-11.mi	M	70	Non-vaccinated	Apr 29, 2020	
SARS-12.mi	M	61	Non-vaccinated	Apr 29, 2020	

(Continues)

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TABLE 1 (Continued)

Patient/sample ID	Gender	Age at the time of sample collection (years)		Date of sample collection	Days between Infection and sample collection
SARS-13.mi	M	57	Non-vaccinated	Apr 30, 2020	
SARS-14.mi	M	60	Non-vaccinated	May 2, 2020	
SARS-15.mi	M	46	Non-vaccinated	May 2, 2020	
SARS-16.mi	M	49	Non-vaccinated	May 2, 2020	
SARS-17.mi	M	39	Non-vaccinated	May 15, 2020	
SARS-18.mi	M	60	Non-vaccinated	Jun 2, 2020	
SARS-19.mi	M	44	Non-vaccinated	Jun 15, 2020	
SARS-20.mi	M	61	Non-vaccinated	Jun 25, 2020	

[a]Indicate SARS-CoV-2 infection.

incubated for 30 min at 37°C. The final serum dilutions ranged from 1:12.5 to 1:12 800. After 30 min, the formed immune complexes were seeded on top of BHK cells expressing FcgRIIa (50 000 cells/well) in 100 µL DMEM high-glucose medium without FBS and incubated for 60 min at 37°C. Subsequently, the medium was replaced with medium with 10% FBS (200 µL), and cells were incubated for 48 h at 37°C. After the incubation, the medium was removed, and cells were washed before being lysed with 50 µL 1X lysis buffer (Bio-Glo™ Luciferase Assay System) and incubated for 20 min. Subsequently, 50 µL substrate (Bio-Glo™ Luciferase Assay System) was added to each well, and the luminescence was measured with a TECAN infinite F200 PRO plate reader.

2.5 | Flow cytometric antigen bead array

Cross-reactive antibodies to SARS-CoV-2 S, receptor-binding domain (RBD), and MERS-CoV S1 were detected with a bead array comprising 11 carboxymethylated beads with 11 different fluorescence intensities from Spherotech (UV-excitable dye). The assay was performed as described elsewhere,[37] with necessary modifications. The bead sets were individually coupled to histidine-tagged recombinant human coronaviruses (hCoV) proteins expressed in human cells (Acro Biosystems). SARS-CoV-2 proteins or protein fragments, including S1, RBD of SARS-CoV-2, and S1 of MERS-CoV, were included in the analysis. The coupling procedure was performed as previously described,[38] and necessary modifications were made to the reconstituted lyophilized recombinant proteins with PBS (pH 7.4) with Zeba columns (Pierce).

Serum samples were diluted 1:20 with assay buffer consisting of 10 mM Tris-HCl (pH 7.5), 0.1% BSA, and 0.01% Tween-20. The diluted serum samples were added to the bead array (2000 microspheres/peak) in a total volume of 50 µL in a Multiscreen HV filter plate and incubated for 35 min at room temperature under agitation at 800 rpm. The microplates were vacuum washed three times with assay buffer and incubated for 20 min in assay buffer containing PE-labeled goat anti-human IgA polyclonal antibodies (0.63 µg/mL; Jackson Immuno Research, 109-115-011), Alexa-Fluor 488-labeled goat anti-human IgA polyclonal antibodies (0.6 µg/mL; Southern Biotech, 2020-31), or Alexa-Fluor 647-labeled goat anti-human IgM polyclonal antibodies (1.2 µg/mL; Southern Biotech, 2020-31) at room temperature under agitation at 800 rpm. The microspheres were then vacuum-washed three times with 10 mM Tris-HCl (pH 7.5) buffer and 0.05% Tween-20, resuspended, and analyzed on a BD FACTA Symphony A5 flow cytometer equipped with violet (450 nm), UV (355 nm), red (633 nm), and yellow–green (561 nm) laser and a high throughput Sampler.

For the analysis, beads were classified with 11 gates in bivariate plots of UV 515 (excitation 355 nm, emission 515/30 nm) fluorescence and violet 525 nm (excitation 405 nm, emission 525/50 nm) fluorescence. The fluorescence intensity revealing populations of beads at each gate was analyzed to determine the level of total IgG and IgG subclasses. The color intensity was B-520 for Alexa-Fluor 488 (excitation 488 nm, emission 525/50 nm), YG-586 for PE (excitation 561 nm, emission 586/14 nm), and R670 for Alexa-Fluor 647 (excitation 640 nm, emission 670/30 nm). FlowJo software was used for the analysis of data in each region, data for an average of 300 beads were captured and analyzed. The positivity index for each sample was calculated from each bead peak with the median fluorescence intensity (MFI) in the fluorescence channels (ratio of case MFI/pooled negative control MFI for the same antigen/isotype). The pooled negative controls were captured from the samples collected and stored before the COVID-19 pandemic.

2.6 | Neutralization assays

The neutralizing antibody levels in serum samples were analyzed using both SARS-CoV-2 and MERS-CoV PVs. HEK293T cells expressing the ACE-2 receptor and Huh7.5 cells expressing the

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DPP4 receptor were used to assess the percentage inhibition (viral entry) of SARS-CoV-2 and MERS-CoV PVs, respectively. Heat-inactivated serum samples were serially diluted into six twofold serial dilutions (in duplicates) starting from 1:50 to perform neutralization assays as previously described.[39] The immune complex was the added to ACE-2 and/or DPP4 expressing cells (50 000 cells/well) and incubated for 48 h. The luminescence was measured with a TECAN infinite F200 PRO plate reader. The percentage neutralization was calculated for each sample dilution against the luminescence of non-serum controls (PVs concentration = 1×10^6 RLU/mL).

2.7 | Statistical analysis

All statistical analyses were performed in Microsoft Excel 2016 and GraphPad Prism 9. One-way ANOVA was used to analyze the differences between groups, and a p value less than 0.05 was considered statistically significant.

3 | RESULTS

This study characterized ADE in 40 samples from 36 patients exposed to two betacoronaviruses, MERS and SARS-CoV-2, through infections and/or vaccination (Table 1). Samples were obtained from patients with MERS infection history without SARS-CoV-2 vaccination ($n = 12$), acute MERS infection ($n = 2$), and COVID-19 infection either severe ($n = 10$) or mild ($n = 10$). Six MERS patients were vaccinated with the SARS-CoV-2 mRNA vaccine (BNT162b2 or mRNA-1273) ($n = 6$), and four of them (MERS 1–4 in Table 1) had serial samples taken before and after vaccination. We also characterized levels of IgG subclasses as well as neutralization activities in these samples, in correlation with ADE activity.

3.1 | Characterization of stable FcgRIIa expression on BHK cells

BHK cells with stable expression of the antibody (IgG) receptor FcgRIIa were characterized with immunofluorescence assays. Immunostaining of these cells revealed the presence of FcgRIIa on transfected cells but not on normal BHK cells (control) (Figure S1).

3.2 | Assessment of ADE activity against SARS-CoV-2

We observed ADE in nine samples (Figures S1A and 3) from patients with a MERS-CoV infection ($n = 16$; Table S3), and only one sample from severely infected COVID-19 patients (Figure 1C). ADE was indicated by a higher luciferase activity when compared to control samples at a dilution 1:3200 which declines in subsequent dilutions. In contrast, other samples, including control samples, showed weak luminescence signals at all dilutions, indicating no ADE activity (Figure 1A–D). There was a significant difference between the peak intensity of luminescence in ADE positive and control samples at lower serum concentration (ordinary one-way ANOVA; $p < 0.0001$). However, we did not observe ADE activity in these samples when using MERS-CoV PVs (Figure S2).

3.2.1 | ADE activity after SARS-CoV-2 vaccination

We then evaluated ADE activity in MERS patients, vaccinated and not-vaccinated against SARS-CoV-2. Due to difficulty in recruitment, we had only four pairs, representing four patients (MERS 1 to MERS 4), of pre- and postvaccination sera. We also had an additional three samples from MERS-infected and SARS-CoV-2-vaccinated patients, one of them was an acute MERS patient with previous exposure to the SARS-CoV-2 vaccine. Using SARS-CoV-2 PVs, the majority of ADE activity was reported in samples of patients who were only exposed to MERS (8 out of 13 [12 convalescent sera and 1 acute sera]) (Figure 2A). In serial samples isolated from patients before and after vaccination, ADE activity was observed in two not-vaccinated samples only (Figure 2B), vaccinated samples did not show any ADE activity (Figure 2C). After vaccination against SARS-CoV-2, ADE was not observed in MERS infected convalescent serum samples.

3.2.2 | ADE activity in acute MERS infection

We also analyzed ADE against SARS-CoV-2 in serial samples from two MERS patients during the acute phase of the disease. From Patient 1 (MERS-15.ai; 50 years old, survived) we had samples from 3, 5, 10, 13, 16, and 23 DPI. From Patient 2 (MERS-16.ai + SARS-CoV-2v; 85 years old, deceased), we had samples from 13 to 17 DPI. The latter was formerly vaccinated against COVID-19. Regardless of the timepoint, both patients reported high levels of ADE activity over pre-pandemic control samples(Figure 3), indicated by ** ($p = 0.0298$), which was higher in Patient 1 compared to Patient 2; however, the difference was not significant ($p = 0.0752$).

We analyzed the average luminescence in serum samples with MERS infection history (MERS.i), MERS acute infection (MERS.ai), MERS infection with SARS-CoV-2 vaccination (MERS.i + SARS-CoV-2v), SARS-CoV-2 severe infections (SARS-CoV-2.si), and SARS-CoV-2 mild infections (SARS-CoV-2.mi). Higher luminescence (ADE) was observed in MERS-infected samples; however, lower luminescence was observed in samples after vaccination (Figure S3).

3.3 | Association of ADE with IgG subtypes and neutralization

We then characterized the levels of IgG (total and subtypes) among ADE positive ($n = 9$), ADE negative ($n = 7$), and control (prepandemic; $n = 6$) groups. We used an antigen bead array to measure IgG

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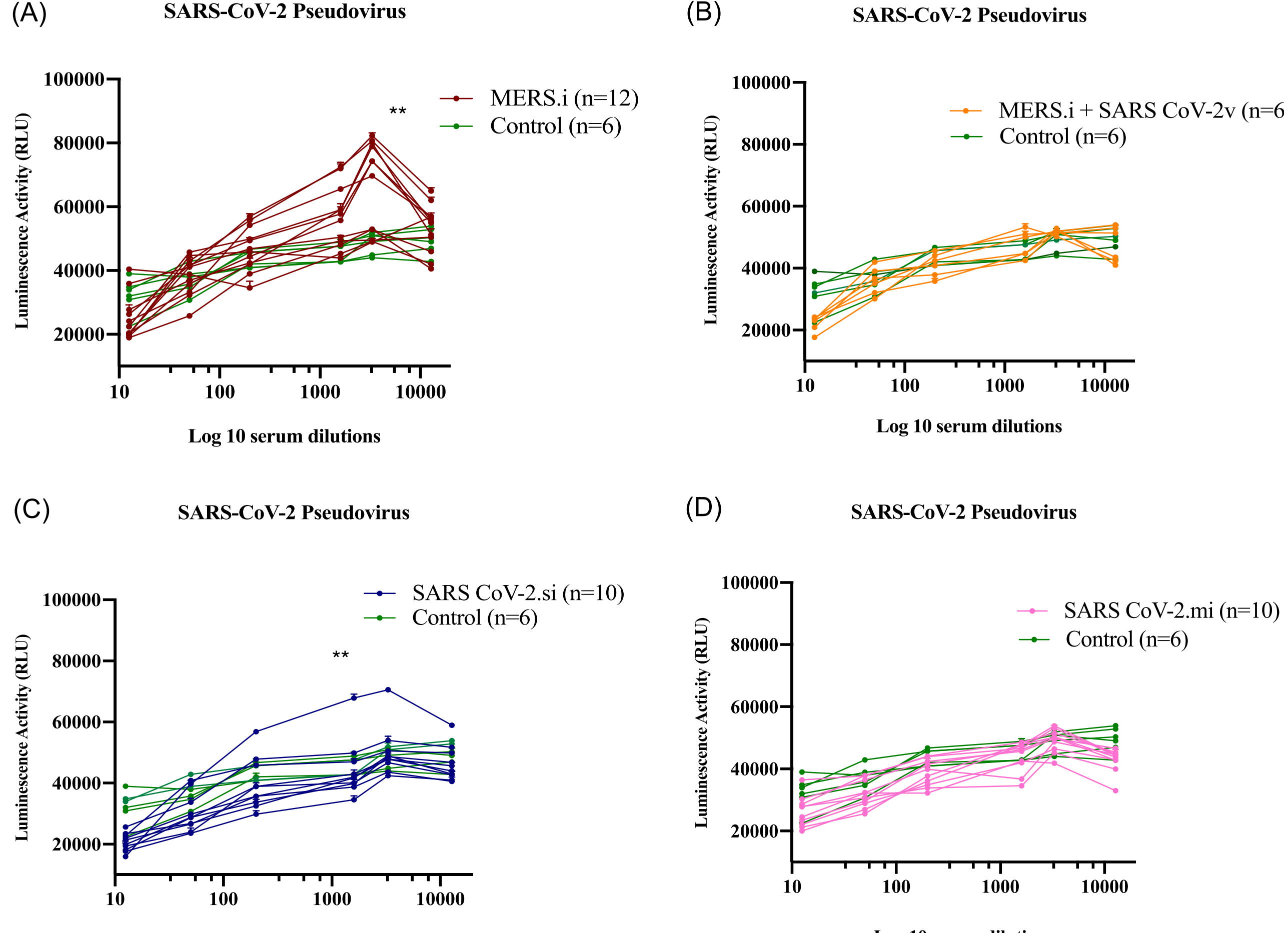

FIGURE 1 ADE against SARS-CoV-2 PVs in sera samples from MERS-infected, SARS-CoV-2-infected, and SARS-CoV-2-vaccinated individuals. ADE (higher luminescence) was reported as luciferase activity inside cells upon uptake of the PVs via the FcγRIIa receptor. Samples represented MERS convalescent sera (n = 12) (A), MERS infection with SARS-CoV-2 vaccination (n = 6) (B), SARS-CoV-2 severe infections (n = 10) (C), and SARS-CoV-2 mild infections (n = 10) (D).

responses against SARS-CoV-2 RBD/S1 and MERS-CoV S1 antigens. Serum IgG levels are significantly lower in the ADE-positive samples compared to ADE-negative samples. The total IgG was significantly lower against all three antigens (p values: SARS-CoV-2 RBD = 0.0036, SARS-CoV-2 S1 = 0.0061, and MERS-CoV S1 = 0.015). Furthermore, we observed lower levels of IgG1, IgG2, and IgG3 against SARS-CoV S1 and RBD in ADE-positive samples ($p < 0.0001$). Also, the levels of IgG1 and IgG2 against MERS S1 antigens were low in ADE-positive samples ($p = 0.024$ in IgG1 and 0.0034 in IgG2). p Values were calculated using one-way ANOVA (Figure 4A–C).

Neutralization activity against MERS-CoV S PVs and SARS-CoV-2 S PVs was assessed in ADE-positive and negative samples at the lowest dilution (1:50). We observed significantly lower levels of neutralizing response, against MERS-CoV PVs and SARS-CoV-2 PVs in ADE-positive samples compared to ADE-negative samples ($p = 0.0041$; 0.0141, respectively) (Figure 4D,E). However, following vaccination, we observed an increase in the neutralizing activity against both viruses, regardless of the ADE status (Figure 4D,E). The increase in the neutralizing activity against MERS-CoV after vaccination with SARS-CoV-2 indicates the elicitation of cross-reactive antibodies.

We then analyzed the association of neutralizing antibody levels with ADE activity in ADE-positive and negative samples. The percent infection/ADE (entry of PVs into BHK-FcgRIIa cells) and inhibition/Neutralization (inhibition of PVs into HEK-293T-ACE-2 cells) were calculated against the RLU of the non-serum controls and the average percent-infection and neutralization were compared between ADE positive and negative samples. We observed a clear pattern of increase in ADE activity with the decreasing level of neutralizing antibodies. Higher ADE levels were observed at a point when the antibody concentration reached below the neutralizing threshold (Figure 5).

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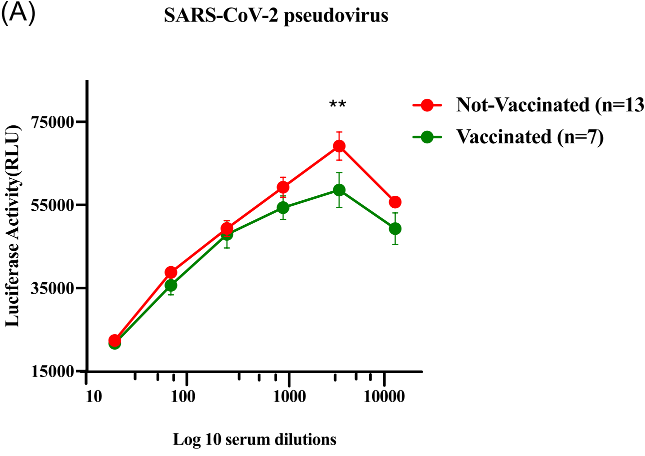

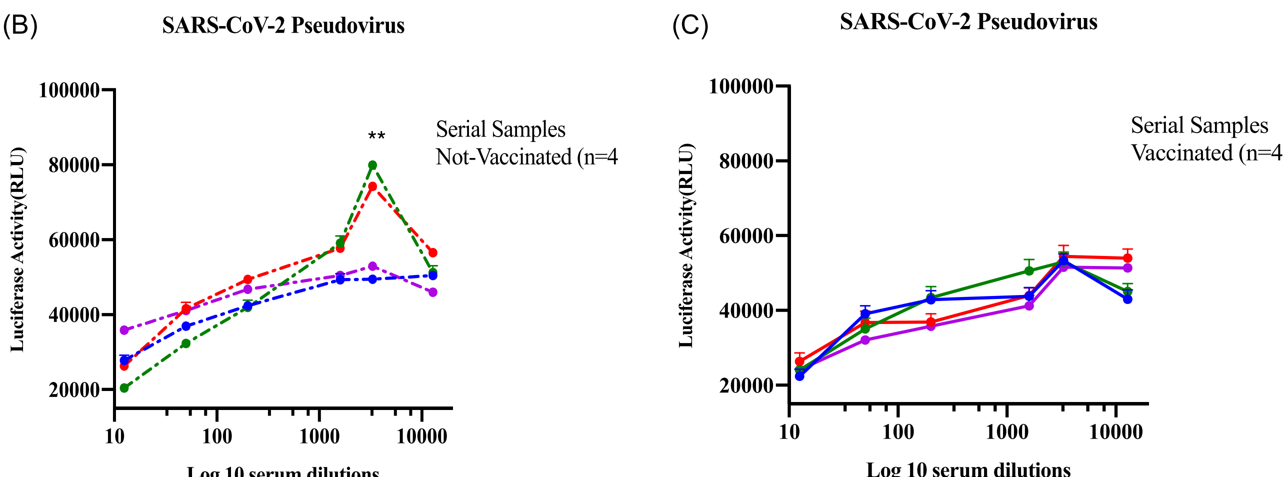

FIGURE 2 ADE of SARS-CoV-2 PVs in samples from MERS-infected patients before and after SARS-CoV-2 vaccination; 20 MERS infection samples (13 not-vaccinated and seven vaccinated samples from 16 patients) (A), serial samples before and after vaccination from four MERS patients (B, C); not-vaccinated (B) and vaccinated (C). Significance is indicated by **.

4 | DISCUSSION

During the past two decades, three outbreaks of betacoronaviruses emerged, resulting in health and social issues globally.[40] Most MERS cases have been reported in the Arabian Peninsula.[33] Given the potential for MERS epidemic/pandemic to occur, the high case-fatality rate (36%),[32] and the sequence similarity between MERS and SARS-CoV-2 (54%), it is crucial to investigate the roles of pre-existing antibodies against MERS-CoV in the disease pathogenesis of other hCoVs including SARS-CoV-2. Pre-existing antibodies against a coronavirus that is non-neutralizing or at sub-neutralizing concentrations may induce ADE after infection with another similar coronavirus.[40] This study investigated the activity of ADE in serum samples from MERS-infected patients, with or without exposure to SARS-CoV-2 antigens.

Using PVs to measure antigen entry to BHK cells expressing FcgRIIa, ADE was reported with SARS-CoV-2 PV but not MERS-CoV PV. Enhanced cell entry of PVs was observed at higher serum dilutions, which is consistent with previous studies.[21,24]

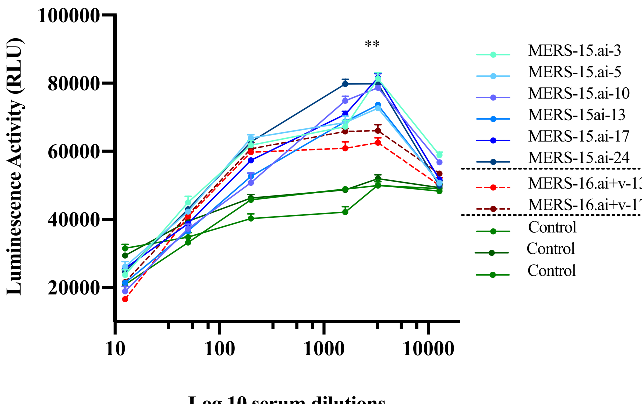

FIGURE 3 ADE in samples from patients with acute MERS infection. Luciferase activity was detected in samples from two ICU MERS patients: MERS-15.ai, survived (3, 5, 10, 13, 17, and 24 days postinfection [DPI]); and MERS-16.ai + SARS-CoV-2v, deceased (13 and 17 DPI). *p* Value significance between patients and controls is indicated by **.

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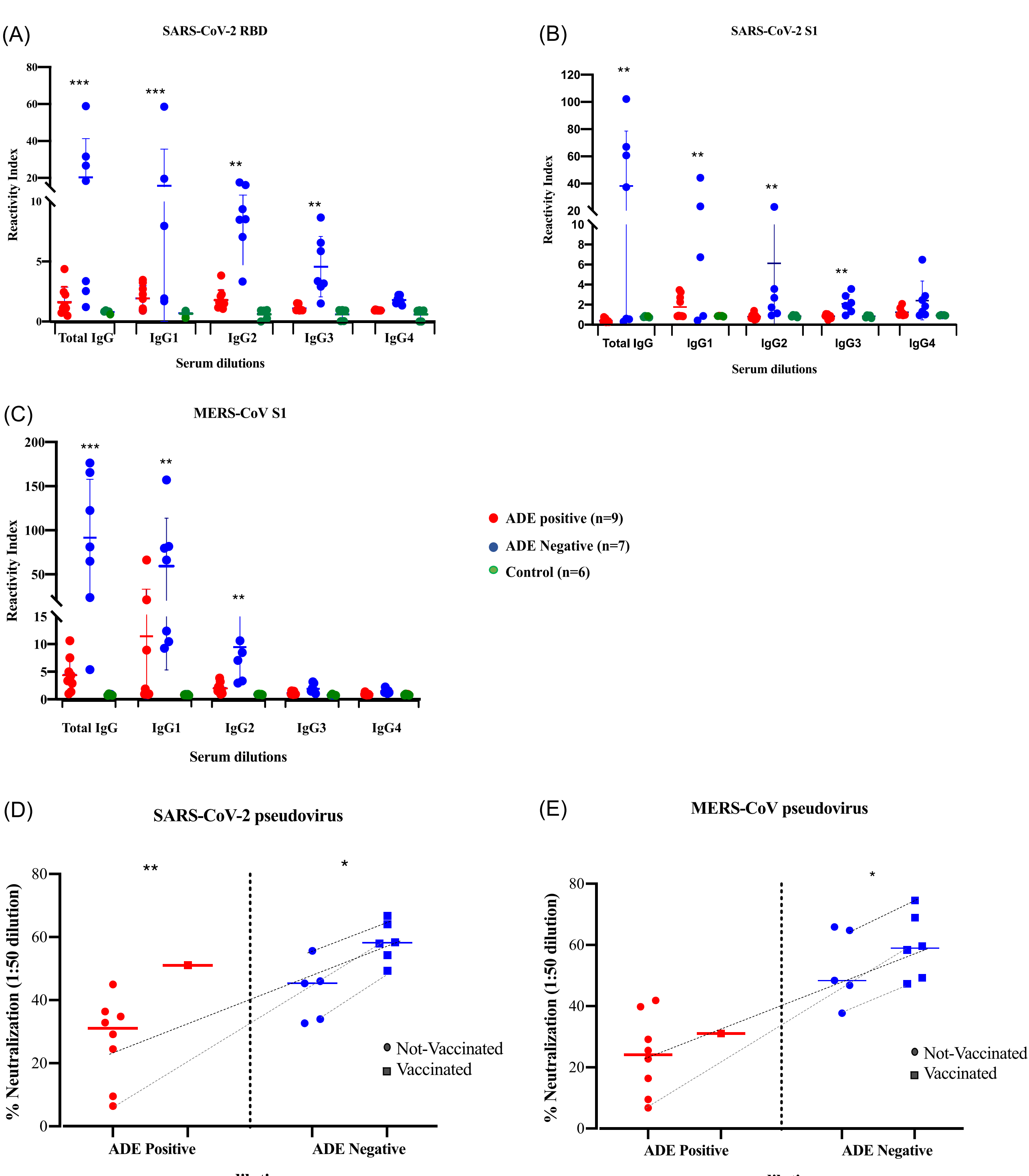

FIGURE 4 IgG responses and neutralization in ADE positive and negative samples. Total IgG and IgG1–4 levels against SARS-CoV-2 RBD (A), SARS-CoV-2 S1 (B), and MERS-CoV S1 (C) antigens. *p* Values were calculated with a one-way ANOVA test and significance is marked in *** or **. Percentage neutralization in ADE positive and negative samples before and after SARS-CoV-2 vaccination (D, E). Neutralization against SARS-CoV-2 PVs (D), MERS-CoV PVs (E) at 1:50 dilution. *p* Values were calculated using one-way ANOVA, and the significance is indicated by ** or *. The serial samples (not-vaccinated and vaccinated) are connected by lines.

Specific attention was drawn to measuring ADE in recovered MERS patients following COVID-19 vaccination. Interestingly, a substantial level of ADE was observed in sera from patients only exposed to MERS. From the 13 MERS-infected samples (12 convalescent and 1 acute) that were not yet exposed to SARS-CoV-2 vaccination, eight samples (61.5%) demonstrated ADE, whereas only one of the seven vaccinated samples demonstrated ADE, knowing that this sample was from the deceased MERS-infected (85 years) patient who was previously vaccinated for

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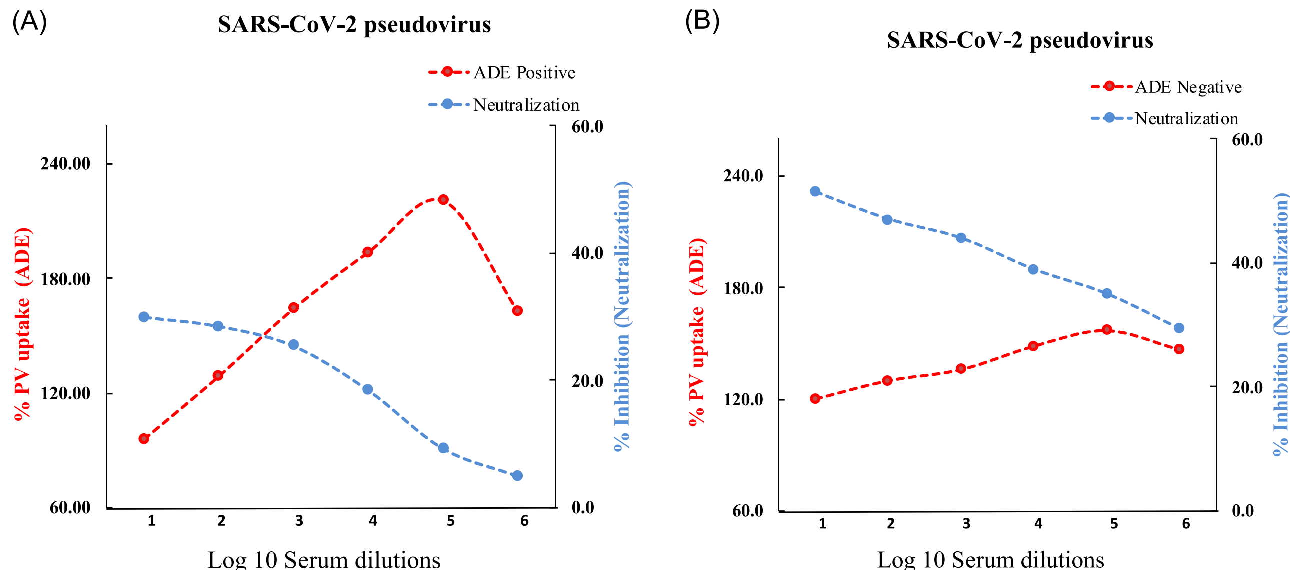

FIGURE 5 Association between ADE and neutralization in (A) ADE-positive ($n = 9$) and (B) ADE-negative ($n = 11$) samples. Percentage of virus uptake (ADE) were calculated as (luminescence of serum dilutions/luminescence of pre-pandemic control) × 100. Similarly, percentage neutralization were calculated as (luminescence of serum dilutions/luminescence of non-serum control) × 100. Average of percent virus uptake and percent neutralization for all samples at designated dilutions were calculated and plotted accordingly.

SARS-CoV-2. Interestingly, of the four patients for whom we had samples before and after COVID-19 vaccination, two patients lost ADE in their serum samples following vaccination. Hence, ADE activity seems to diminish in MERS patients following SARS-CoV-2 vaccination, and instead, broad neutralizing activity (to MERS-CoV and SARS-CoV-2) is generated. Interestingly, only one patient, 85 years old (deceased), was first vaccinated with SARS-CoV and then infected with MERS-CoV. This patient also demonstrated ADE activity, at different time points, in his sera after MERS infection. However, we did not have per-exposure sera to see whether this activity was already there or it was generated due to MERS infection. Nonetheless, the overall picture denotes that MERS infection is associated with elicitation of ADE activity against SARS-CoV-2. This was reported in both convalescent sera and sera collected from acute patients. Several papers indicated that pre-existing immunity could shape the immune response to SARS-CoV-2 harmfully or beneficially.[41] Hence, the order of exposure to different CoV may lead to ADE response in some individuals. However, this is not very clear in our case considering the low sample size, and further studies with a higher number of samples are needed.

On the other hand, cross-neutralization between beta CoV has been reported. we recently reported that patients exposed to MERS-CoV and SARS-CoV-2 antigens mount broadly reactive responses that target other human CoVs as well.[39] Similarly, another recently published study reported neutralization of SARS-CoV-2 by camel-generated MERS antibodies.[37] Using human and mouse sera, Lv et al. reported that cross-reactivity between beta-CoV is possible.[31] This is entitled to the boosting of cross-reactive antibodies that target neutralizing epitopes. Identifying these epitopes is crucial for the structural design of vaccines that strictly elicit cross-neutralizing antibodies while diminishing harmful responses.

Most of the ADE studies were conducted in the context of SARS-CoV and SARS-CoV-2 which seems to be similar between the two viruses.[42] We are the first group to report high ADE activity in MERS patients. However, we lack an explanation of how and why this response is generated. Enhanced entry of MERS-CoV PV into FcR (CD16A, CD32A, and CD64A) expressing HEK293T cells was reported using Mersmab1 MERS monoclonal antibody, confirming Fc receptor-dependent ADE pathway.[43] This study supports our findings that ADE could occur in individuals with pre-existing immunity to MERS infection, resulting in severe infection in some patients.

It is worth noting that our samples were collected from confirmed MERS patients with respiratory illness. However, multiple studies reported that MERS infection can go unnoticed and with minimal symptoms.[44] Hence, it would be interesting to compare ADE activity in asymptomatic and symptomatic MERS patients and to decipher the role of pre-existing to other human CoV in shaping disease pathogenesis.

Antibody subclasses and the epitopes they target may play a role in defining ADE activity in some patients. Hence, we analyzed IgG subtypes against one MERS-CoV (S1) and two SARS-CoV-2 (RBD and S1) antigens in ADE-positive and ADE-negative samples. Total IgG against all three antigens was significantly low in ADE-positive samples. Similarly, IgG1, IgG2, and IgG3 subclasses against SARS-CoV-2 antigens were also low in ADE-positive samples. On the other hand, anti-MERS-CoV S1 IgG1 and IgG2 subclasses were lower in the ADE-positive samples. Overall, we observed that ADE is associated with lower IgG levels (consistently IgG1 and 2) against CoV surface antigens. Previous in vitro studies using serum samples from COVID-19-infected samples also reported similar findings that cross-reactive but non-neutralizing antibodies can be the cause of ADE.[31,45] Expectedly, a lower level of IgG against surface

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antigens translates into lower neutralization activity in ADE-positive samples. Studies reported that MERS-CoV and SARS-CoV-2 are ~40% similar in their S protein, which is the main target for neutralizing antibodies. Typically, the S protein is more conserved in the S2 domain compared to S1/RBD among beta-CoV.[46] Previous studies showed that SARS-CoV-2 neutralizing antibodies are more directed to the neutralizing epitopes of N terminal domain and RBD of the S1 subunit. These epitopes of S1 are more capitulated to selective pressure and hence increase the possibility of immune escape by mutants.[46] However, broadly neutralizing antibodies seem to target the S2 subunit, which is more conserved. In our study, we only used S1/RBD subunits in the analysis. However, it would be crucial to characterize the targeted epitopes in samples with ADE in comparison to those with broadly neutralizing activity.

5 | CONCLUSION

We observed a high rate of ADE activity in convalescent sera from MERS patients, against SARS-CoV-2 but not MERS-CoV PVs. ADE activity was diminished in individuals exposed to both antigens. Interestingly, vaccination of MERS patients with the SARS-CoV-2 mRNA vaccine induced a broadly neutralizing response in some patients. The ADE activity was associated with low IgG levels and neutralizing activity. The low sample size in our study refers to the low number of MERS cases worldwide and the difficulty in approaching these patients. However, this study raises several questions to be answered, importantly, the identification of targeted epitopes by ADE antibodies, as that would aid in structural-based vaccine design. This study evaluated ADE in the context of MERS-CoV and SARS-CoV antigens only. Further studies shall consider a similar analysis in the context of pre-existing immunity to other human CoV.

AUTHORS CONTRIBUTIONS

Hadi M. Yassine and Swapna Thomas conceptualized and designed the study. Swapna Thomas did the analysis. Swapna Thomas, Maria K. Smatti, Hadeel T. Zedan, Hadi M. Yassine, and AAJA contributed to sample collection, processing, and testing. Jean-Charles Grivel and Giusy Gentilcore conceived and conducted the hCoV antigen bead array immunoassays. Ali H. Eid, Ali A. Hssain, Gheyath K. Nasrallah, Asmaa A. Althani, and Hadi M. Yassine provided resources. Hadi M. Yassine oversaw the study. Allal Ouhtit supervised the student academically. Hadi M. Yassine and AAJA provided funding. Swapna Thomas wrote the initial draft. Haya Alsulaiti and Hadi M. Yassine reviewed and edited the manuscript. All others have contributed to data and sample gathering, and manuscript editing.

ACKNOWLEDGMENTS

We thank Yoshihiro Kawaoka et al. for providing BHK cells expressing FcgRIIa. We also thank the Virology Research Center at the National Institutes of Health for providing the plasmids used to generate SARS-CoV-2 PVs and for providing HEK293T cells. This study was supported by partial funds from QU grant no. QUCG-BRC-2022/23-578.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All the data that support the findings enclosed in this manuscript is described in the manuscript and the same is available upon request.

ETHICS STATEMENT

Ethical approval for sample collection and processing was obtained from Qatar University (QU-IRB-1289-EA/20), the Qatar Biobank Institutional Review Board (QF-QBB-RES-ACC-0184), and Hamad Medical Corporation (HMC)(MRC-01-20-145).

ORCID

Hadeel T. Zedan http://orcid.org/0000-0002-9484-7290
Laith J. Abu Raddad http://orcid.org/0000-0003-0790-0506
Gheyath K. Nasrallah http://orcid.org/0000-0001-9252-1038
Hadi M. Yassine http://orcid.org/0000-0001-7592-2788

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How to cite this article: Thomas S, Smatti MK, Alsulaiti H, et al. Antibody-dependent enhancement (ADE) of SARS-CoV-2 in patients exposed to MERS-CoV and SARS-CoV-2 antigens. *J Med Virol*. 2024;96:e29628. doi:10.1002/jmv.29628

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