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Characterization of neutralizing versus binding antibodies and memory B cells in COVID-19 recovered individuals from India

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ABSTRACT

India is one of the most affected countries by COVID-19 pandemic; but little is understood regarding immune responses to SARS-CoV-2 in this region. Herein we examined SARS-CoV-2 neutralizing antibodies, IgG, IgM, IgA and memory B cells in COVID-19 recovered individual from India. While a vast majority of COVID-19 recovered individuals showed SARS-CoV-2 RBD-specific IgG, IgA and IgM antibodies (38/42, 90.47%; 21/42, 50%; 33/42, 78.57% respectively), only half of them had appreciable neutralizing antibody titers. RBD-specific IgG, but not IgA or IgM titers, correlated with neutralizing antibody titers and RBD-specific memory B cell frequencies. These findings have timely significance for identifying potential donors for plasma therapy using RBD-specific IgG assays as surrogate measurement for neutralizing antibodies in India. Further, this study provides useful information needed for designing large-scale studies towards understanding of inter-individual variation in immune memory to SARS CoV-2 natural infection for future vaccine evaluation and implementation efforts.

1. Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the virus responsible for the coronavirus disease 2019 (COVID-19) pandemic, emerged as a grave public health threat beginning in December 2019 (WHO, 2020), paralyzing daily lives and causing economic downturns in many parts of the world. Currently, India is one of the countries most affected with more than 10.6 million COVID-19 confirmed cases and 155,732 associated deaths as of February 16, 2021 (Ministry of Health and Family Welfare GoI, 2020).

Intense efforts are underway to develop vaccines and antiviral therapeutics (Al-Kassmy et al., 2020; Amanat and Krammer, 2020; Dagotto et al., 2020; Hashemian et al., 2020; Li and Kang, 2020; Malik et al., 2020; Nabil et al., 2020; Parvathaneni and Gupta, 2020; Verma

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et al., 2020). All these efforts require a detailed understanding of immune correlates of protection, formation of immune memory, and durability of these responses. Additionally, infusion of plasma derived from COVID-19 recovered individuals is being explored as a management strategy (Bloch, 2020; Dhanasekaran et al., 2020; Duan et al., 2020; Focosi et al., 2020; Rabelo-da-Ponte et al., 2020; Salazar et al., 2020; Shen et al., 2020; Ye et al., 2020; Ju et al., 2020) and its rate of success will be augmented with a better understanding of humoral immunity, immunoglobulin isotype usage and neutralizing activity following recovery from SARS-CoV-2 infection. Moreover, given that many of the SARS-CoV-2 neutralizing epitopes are located in the viral receptor binding domain (RBD) of the spike (S) protein (Barnes et al., 2020; Cao et al., 2007; Lan et al., 2020; Liu et al., 2020a; Mittal et al., 2020; Peterhoff et al., 2020; Wang et al., 2020a; Zost et al., 2020a, 2020b), it is important to evaluate the relationship between RBD-specific IgG titers and neutralizing antibody responses in recovered individuals from India.

In this study, we evaluated IgG, IgA, IgM, neutralizing antibodies and memory B cell responses in PCR-confirmed COVID-19 convalescent subjects. Our results show that while a vast majority (38/42, 90.47%) of COVID-19 recovered individuals developed SARS-CoV-2 RBD-specific IgG responses, we were able to detect appreciable levels of neutralizing antibody responses in only half of the convalescent subjects. Neutralizing responses correlated closely with RBD-specific IgG titers, but weakly with IgG titers measured against whole virus concentrate using a commercial Enzyme Linked Immunosorbant Assay (ELISA) kit. Taken together, these findings suggest that despite significant inter-individual variation in the RBD-specific IgG titers and neutralizing antibodies, RBD-specific IgG titers continue to serve as a valuable and robust surrogate measurement for neutralizing antibody responses. Our observations, that many individuals do not develop appreciable levels of neutralizing antibodies, not only provides a glimpse of humoral immune responses in COVID-19 recovered individuals from India, but also has timely implications for identifying potential plasma therapy donors using on RBD-specific IgG ELISA's in India where routine performance of neutralization assays remains a challenge.

2. Methods

2.1. Patient recruitment

COVID-19 recovered individuals were recruited at Shaheed Hasan Khan Mewati Government Medical College, Nuh, Haryana, India, Super Specialty Pediatric Hospital and Post Graduate Teaching Institute, Noida and ICMR-National Institute of Malaria Research, New Delhi. The Institutional ethical boards approved the study. Informed consent was obtained prior to inclusion in the study. All subjects (mean age 39.4 years, range 15–70 years) were SARS-CoV-2 PCR positive at the time of initial diagnosis, and were PCR negative when recruited for this study at 3.6–12 weeks post initial diagnosis (Table 1). Samples collected from healthy adult blood bank donors in the year 2018 are included as pre-pandemic controls.

Table 1
COVID-19 recovered individuals characteristics (n = 42)[a].

Age in years Mean (Range)	39.4 (15–70)
Males/Females	38/4
Days post PCR diagnosis Mean (Range)	47.3 (25–84)

[a] COVID-19 recovered individuals were recruited at Shaheed Hasan Khan Mewati Government Medical College, Nuh, Haryana, India. Super Speciality Paediatric Hospital and Post Graduate Teaching Institute, Noida and ICMR-National Institute of Malaria Research, New Delhi. All subjects were SARS-CoV-2 PCR positive at the time of initial diagnosis and were PCR negative when recruited for this study at 4.8–11 weeks post initial diagnosis.

2.2. SARS-CoV-2 specific PCR

Nasopharyngeal and throat swabs were collected in viral transport medium (VTM) (HiMedia, #AL 167)) and transported to the testing laboratory maintaining cold chain. All the samples were screened by qRT-PCR assay as the standard operating procedures established by Indian Council of Medical Research (ICMR)-National Institute of Virology (NIV), Pune, India under the Government of India guidelines for COVID-19 diagnosis. (ICMR-NIV, 2020).

2.3. SARS-CoV-2 RBD-specific direct ELISA

The recombinant SARS-CoV-2 RDB gene was cloned, expressed, purified and ELISAs were performed as previously described (Suthar et al., 2020). Briefly, purified RBD was coated on MaxiSorp plates (Thermo Fisher, #439454) at a concentration of 1 μg/mL in 100 μL phosphate-buffered saline (PBS) at 4 °C overnight. The plates were washed extensively with PBS containing 0.05% Tween-20. Three-fold serially diluted plasma was added to the plates and incubated at room temperature for 1hr. After incubation, the plates were washed and the SARS-CoV-2 RBD specific IgG, IgM, IgA signals were detected by incubating with horseradish peroxidase (HRP) conjugated - anti-human IgG (Jackson ImmunoResearch Labs, #109-036-098), IgM (Jackson ImmunoResearch Labs, #109-036-129), or IgA (Jackson ImmunoResearch Labs, #109-036-011). Plates were then washed thoroughly and developed with *o*-phenylenediamine (OPD) substrate (Sigma, #P8787) in 0.05 M phosphate-citrate buffer (Sigma, #P4809) pH 5.0, containing with 0.012% hydrogen peroxide (Fisher Scientific, #18755) just before use. Absorbance was measured at 490 nm.

2.4. Enumeration of SARS-CoV-2 RBD-specific memory B cells

Purified RBD protein (100 μg) was labeled with Alexa Fluor 488 using microscale protein labeling kit (Life Technologies, #A30006) as per manufacturer's protocol. PBMC's were stained with RBD-Alexa Fluor 488 for 1 h at 4 °C, followed by washing with PBS containing 0.25% Fetal Bovine Serum (FBS), and incubation with efluor780 Fixable Viability (Live Dead) dye (Life Technologies, #65-0865-14) and anti-human CD3, CD19, CD27, CD38 and IgD antibodies (BD Biosciences) for 30 min. Cells were washed twice with FACS buffer and acquired on BD LSR Fortessa X20. Data was analyzed using FlowJo software 10. SARS-CoV-2 RBD-specific memory B cells were identified in cells positive for CD19, CD20, CD27 that were negative for IgD and CD3.

2.5. IgG ELISA for SARS-CoV-2 whole virus preparation

SARS-CoV-2 antigen specific IgG was detected using a commercially available assay (COVID-Kavach ELISA tests kit, Zydus diagnostics), which measures responses to antigen concentrated from gamma-irradiated SARS-CoV-2-infected tissue culture fluid as per the manufacturer's instructions (Chaudhuri et al., 2020; Sapkal et al., 2020).

2.6. SARS-CoV-2 neutralization assay

Neutralization titers to SARS-CoV-2 were determined as previously described (Suthar et al., 2020). Briefly infectious clone of the full-length mNeonGreen SARS-CoV-2 (2019-nCoV/USA_WA1/2020) was used to test heat-inactivated COVID-19 convalescent samples and healthy donor samples (pre-pandemic). Heat-inactivated serum was serially diluted three-fold in duplicate starting at a 1:20 dilution in a 96-well round-bottom plate and incubated between 750 FFU of SARS-CoV-2-mNG for 1 h at 37 °C. This antibody-virus mixture was transferred into the wells of a 96-well plate that had been seeded with Vero-E6 cells the previous day at a concentration of 2.5×10^4 cells/well. After 1 h, the antibody-virus inoculum was removed and 0.85% methylcellulose in 2% FBS containing DMEM was overlaid onto the cell

monolayer. Cells were incubated at 37 °C for 24 h. Cells were washed three times with 1XPBS (Corning Cellgro) and fixed with 125 μl of 2% paraformaldehyde in PBS (Electron Microscopy Sciences) for 30 min. Following fixation, plates were washed twice with 1x PBS and imaged on an ELISPOT reader (CTL Analyzer). Foci were counted using Viridot (Katzelnick et al., 2018) (counted first under the "green light" setting followed by background subtraction under the "red light" setting). FRNT-mNG$_{50}$ titers were calculated by non-linear regression analysis using the 4 PL sigmoidal dose curve equation on Prism 8 (Graphpad Software). Neutralization titers were calculated as 100% x [1- (average foci in duplicate wells incubated with the specimen) ÷ (average number of foci in the duplicate wells incubated at the highest dilution of the respective specimen).

2.7. Statistical analysis

Statistical analysis was performed using GraphPad prism 8.0 software. Non-parametric *t*-test (Mann-Whitney) was used to calculate the differences between groups. Non-parametric Spearman's correlation coefficient (r) was used to calculate correlation between groups. A *p*

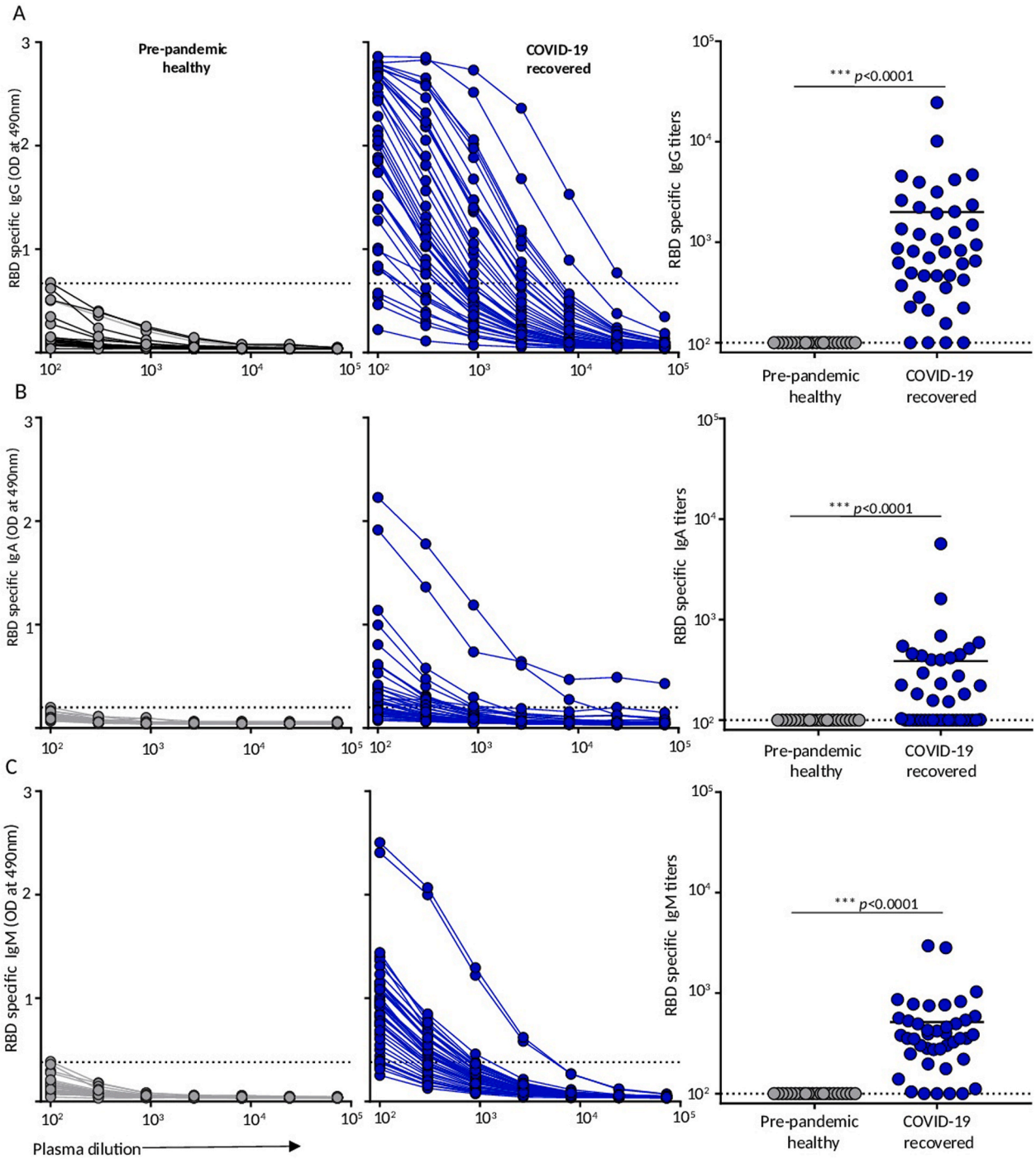

Fig. 1. Evaluation of SARS-CoV-2 RBD specific IgG, IgA and IgM antibody responses. (A) RBD-specific IgG, **(B),** RBD-specific IgA; **(C),** RBD-specific IgM. Left, pre-pandemic healthy (n-22), middle COVID-19 recovered (n = 42); right, endpoint titers. ELISA cutoff values are calculated using the average plus 3 standard deviations of the 22 healthy controls at 1:100 dilution (shown as a dotted line). The unpaired analysis was done using non-parametric Mann-Whitney-U test. $p \leq 0.05$ was considered significant. Assay cutoff value is marked with dotted line.

value of ≤0.05 was considered as significant.

3. Results

3.1. SARS-CoV-2 RBD-specific humoral immunity in COVID-19 recovered individuals

The demographic profile of COVID-19 recovered individuals recruited for this study is shown in Table 1. All subjects were at least 3.6 weeks past their initial SARS-CoV-2 positive diagnosis. RBD-specific ELISA curves for IgG, IgA and IgM at different dilutions of plasma in pre-pandemic healthy versus COVID-19 recovered individuals is shown in Fig. 1. RBD-specific responses were highly elevated in COVID-19 recovered individuals as compared to pre-pandemic healthy controls (Fig. 1A,B,C**, left versus middle panels)**. Titers of IgG, IgA and IgM in the COVID-19 recovered individuals showed substantial inter-individual variation (Fig. 1 A, B, C**, right panel)** - with IgG endpoint titers ranging from below detection to 24,484 (2000 ± 619); IgA titers from below detection to 5686 (386 ± 136) and IgM titers from below detection to 2958 (515 ± 90). Four individuals had undetectable RBD-specific IgG and IgA titers. One of these individuals was also below detection for IgM (Table 2). Inter-individual heterogeneity was not related to the age of the individuals (Fig. 2A) or the number of days that elapsed between PCR confirmation of infection and sample collection (Fig. 2B).

3.2. SARS-CoV-2 specific neutralizing titers in COVID-19 recovered individuals

To assess plasma neutralizing titers from COVID-19 convalescent individuals, we performed a live virus neutralization assay using a focus-reduction neutralization mNeonGreen (FRNT-mNG) assay (Suthar et al., 2020). The neutralizing activity at different dilutions of plasma for pre-pandemic healthy individuals (Fig. 3A) and COVID-19 recovered individuals is shown in (Fig. 3B). Fig. 3C shows FRNT-mNG$_{50}$ titers calculated based on the plasma dilution that neutralized 50% of the virus. While all pre-pandemic healthy individuals had undetectable FRNT-mNG$_{50}$ titers, only half of the COVID-19 recovered individuals showed 50% or more neutralization even at a 1:20 dilution of plasma. Similar to RBD-specific IgG titers, the FRNT-mNG$_{50}$ titers were heterogeneous with the latter reaching titers as high as 682 (Fig. 3C).

Table 2
Individual characteristics of the COVID-19 recovered subjects.

Subject number	Age	Gender (Male,M Female,F)	Days Post PCR Diagnosis	SARS CoV-2 RBD specific Immunoglobulin titers[a]			SARS Cov-2 whole Virus specific IgG ELISA values[b]	Neutralization titer (FRNT-mNG$_{50}$)[c]
				IgG	IgM	IgA		
1	23	M	84	2220	565	220	26	39
2	22	F	84	354	283	<100	3	26
3	68	M	40	464	<100	<100	19	<20
4	35	M	51	4547	393	545	6	113
5	50	M	37	1354	301	275	7	81
6	29	M	34	<100	866	<100	<1.5	<20
7	27	M	34	422	104	450	<1.5	<20
8	25	M	34	222	1031	<100	26	<20
9	21	M	40	650	588	153	9	25
10	39	M	38	612	539	5686	12	23
11	46	M	38	2011	325	224	24	55
12	31	M	38	494	828	183	10	<20
13	20	M	41	944	274	<100	14	49
14	36	M	41	228	279	1614	<1.5	<20
15	34	M	44	282	302	<100	4	<20
16	70	M	44	1250	220	518	14	43
17	40	M	45	464	112	101	16	<20
18	32	M	41	867	381	399	<1.5	<20
19	57	M	45	1069	354	231	<1.5	<20
20	27	F	49	1935	528	<100	23	80
21	36	M	49	3156	355	593	28	166
22	24	M	45	<100	387	<100	<1.5	<20
23	55	F	45	<100	778	<100	<1.5	<20
24	15	M	45	212	496	<100	<1.5	<20
25	49	M	45	4183	2958	397	17	657
26	26	M	48	2352	<100	<100	16	48
27	54	F	54	1202	<100	182	15	49
28	53	M	52	799	197	417	12	<20
29	52	M	48	2611	249	157	23	46
30	45	M	62	1490	401	<100	15	50
31	52	M	56	10,127	421	437	21	434
32	26	M	47	<100	<100	<100	<1.5	<20
33	32	M	57	701	177	<100	14	<20
34	44	M	49	815	428	<100	20	<20
35	32	M	40	829	140	<100	6	29
36	44	M	42	4685	494	295	26	167
37	22	M	77	3954	764	690	24	209
38	49	M	25	24,484	2828	459	22	682
39	55	M	51	371	753	<100	17	<20
40	36	M	51	621	350	104	17	<20
41	60	M	51	156	459	<100	17	34
42	62	M	47	467	354	<100	6	<20

[a] ELISA end point titre limit of detection is 100.
[b] ELISA was performed with a commercial kit (Covid Kavach, Zydus) using 1:100 dilution of plasma as per by the manufacturer's recommendation. Assay cut off is 1.5.
[c] Neutralization titres: Neutralization assay were performed using 3 fold dilution of plasma, starting at 1:20 up to 1:43,740. Limit of detection for FRNT-mNG$_{50}$ is 20.

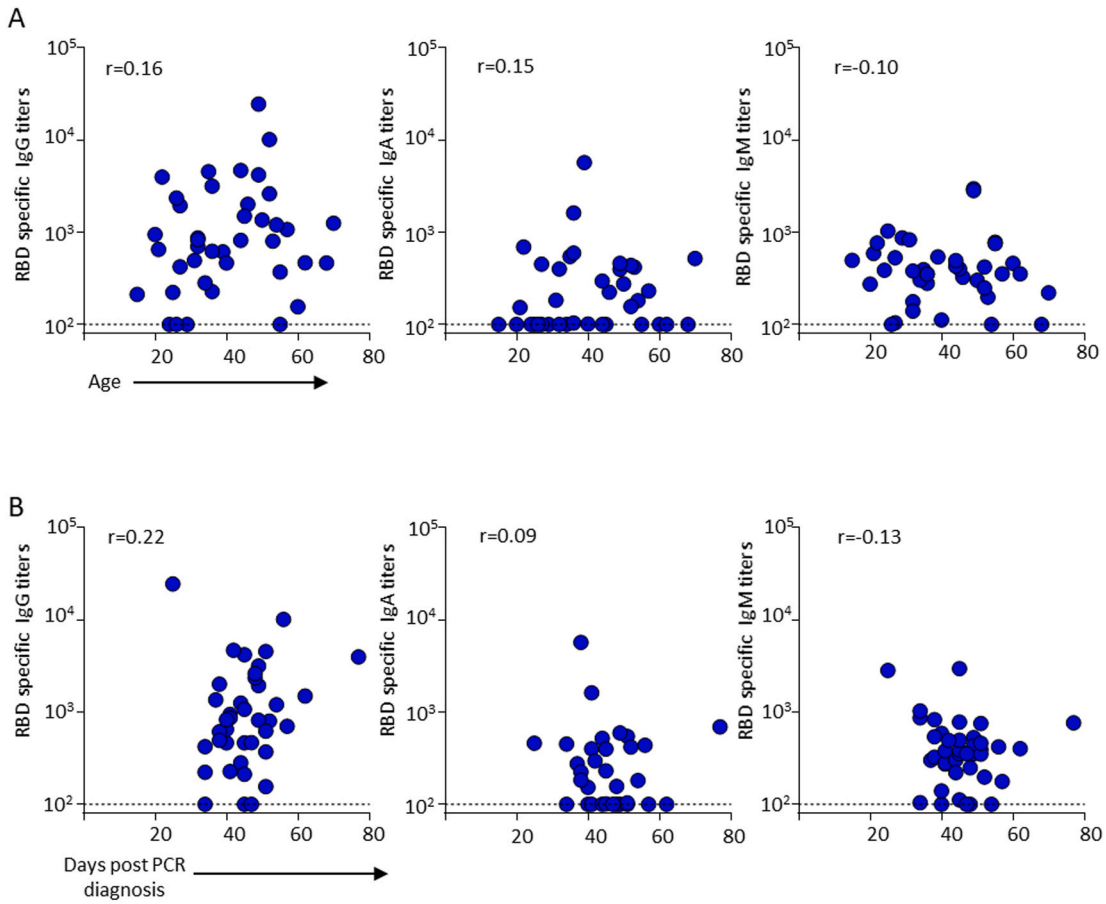

Fig. 2. Correlation of age and day post initial diagnosis of COVID-19 recovered individuals with SARS-CoV-2 IgG, IgM and IgA titers. (A). Age versus IgG (left, n = 42), IgA (middle, n = 42) or IgM (right, n = 42) titers. **(B).** Time post initial diagnosis versus IgG (left, n = 42), IgA (middle, n = 42) or IgM (right, n = 42) titers. Correlations were calculated by Spearman's correlation coefficient r. $p \leq 0.05$ is considered significant. Note that none of the data sets above reached significant values of correlation.

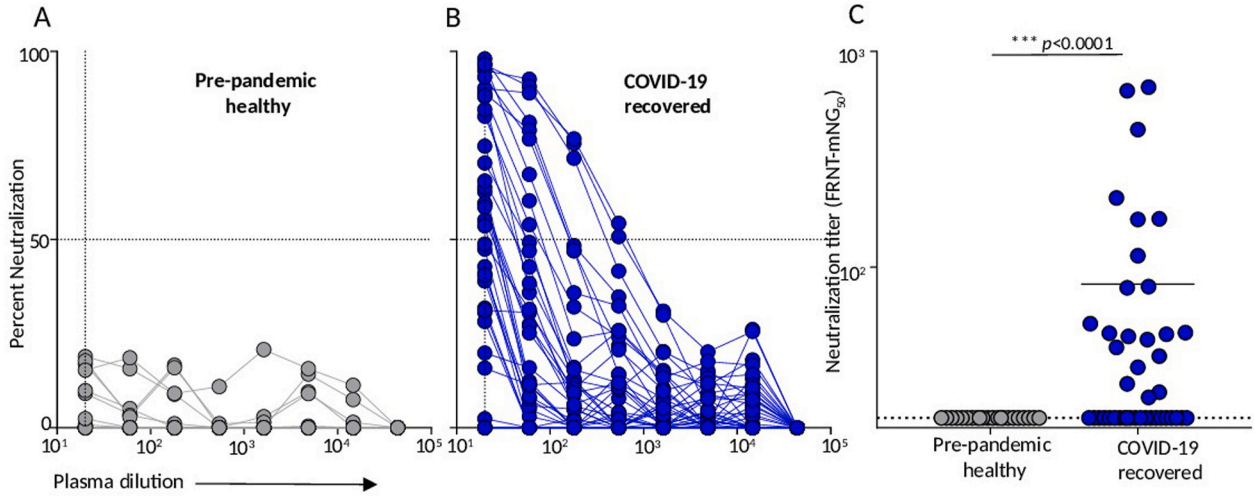

Fig. 3. Evaluation of SARS-CoV-2 neutralizing antibodies in COVID-19 recovered individuals. SARS-CoV-2 neutralizing activity at indicated dilutions of plasma is shown in pre-pandemic healthy (n = 22, in grey) **(A)** and in COVID-19 recovered individuals (n = 42, in blue) **(B)**. Dotted line represents the plasma dilution that leads to 50% neutralization. **(C)** Scatter plot shows neutralization titers (FRNT-mNG$_{50}$) in pre-pandemic healthy (n = 22) and COVID-19 recovered (n = 42) individuals. The unpaired analysis was done using non-parametric Mann-Whitney-U test. $p \leq 0.05$ was considered significant. Limit of detection is marked with a dotted line.

Previous studies in other viral infections have shown that all three antibody isotypes (IgG, IgA and IgM) can potentially neutralize (Chua et al., 2017; Ejemel et al., 2020; Lizeng et al., 2004; Skountzou et al., 2014; Sterlin et al., 2020). We next determined if any correlation exists between SARS-CoV-2 neutralizing titers and RBD-specific IgG, IgA, IgM binding antibody titers. We observed a positive correlation ($r = 0.83$; $p < 0.001$) between SARS-CoV-2 neutralizing titers and RBD-specific IgG titers (Fig. 4, **left graph)** but not with IgA **(**Fig. 4, **middle graph)** or IgM titers (Fig. 4, **right graph)**.

Plasma infusion therapy has recently been started in India as an intervention therapy for COVID-19. For this, plasma donors are being typically identified by the presence of IgG to SARS-CoV-2 by commercial ELISA tests (cdsco.gov.in, 2020). One of these tests detects IgG towards viral antigens concentrated from gamma-irradiated SARS-CoV-2-infected tissue culture fluid (Chaudhuri et al., 2020; Sapkal et al., 2020). It was therefore of interest to examine the correlation between neutralization titers and IgG responses measured using this test. We observed that, of the 42 COVID-19 recovered individuals tested, 33 were IgG positive whereas 9 were below the assay cut off (Fig. 5A). Of the 9 individuals that were below cut off, 4 also tested negative by the RBD-specific IgG ELISA (Table 2). All of the samples from the pre-pandemic healthy individuals were below the limit of detection. As expected, the IgG values obtained by whole virus-based ELISA did not show as robust a correlation ($r = 0.56$) with neutralizing antibody titers (Fig. 5B) as compared to those observed with RBD-specific IgG titers ($r = 0.83$) (Fig. 4, **left graph)**.

3.3. Characterization of RBD-specific memory B cells in COVID-19 recovered individuals

While circulating neutralizing antibodies help prevent re-infection by viruses, memory B cells allow for rapid production of new antibodies in case of re-infection. To address whether the COVID-19 recovered individuals generated memory B cells, we enumerated RBD-specific memory B cells using fluorescently conjugated RBD antigen. An example of the flow cytometric gating strategy and RBD staining among the gated memory B cells is shown in Fig. 6A and B. Fig. 6C shows the frequency of RBD-specific memory B cells in a subset of the individuals where sufficient PBMCs were available. Though we found that there was substantial inter-individual variation in the frequency of SARS-CoV-2 RBD-specific memory B cells, their frequencies modestly correlated with RBD-specific IgG titers.

4. Discussion

Our study provides a succinct analysis of humoral immunity and memory B cells in COVID-19 recovered individuals from India. We examined SARS-CoV-2 neutralizing antibodies, IgG, IgM, IgA and memory B cells in pre-pandemic healthy versus COVID-19 recovered individuals and further evaluated inter-individual variation and relation among these.

Our correlative analysis of RBD-specific IgG binding titers with neutralizing antibody titers and memory B cells corroborates with other studies (Abe et al., 2020; Vaisman-Mentesh et al., 2020; Nguyen-Contant et al., 2020; Tan et al., 2020; Wang et al., 2020b) and has important implications for not only identifying potential donors for plasma therapy but also for understanding humoral and cellular memory formed post COVID-19 recovery from individuals in India. Though current plasma therapy guidelines in India do not consider neutralizing antibody titers, United States Food and Drug Administration (FDA) guidelines recommend, when available, a neutralizing titer of 1:160 or 1:80 to be used for identifying potential plasma donors (FDA, 2020). Our correlation analysis shows that RBD-specific titers of more than 3668 can provide a suitable surrogate for identifying the individuals with neutralizing titers of above 1:160 and RBD-specific IgG titers 1926 for neutralizing titers of 1:80. Recently, in a randomized control trial, 235 COVID-19 patients across 39 clinical sites across India received convalescent plasma therapy to access the role of convalescent plasma in management of COVID-19 (Agarwal et al., 2020). This study did not find any association of reduction in progression to severe COVID-19 or mortality with administration of convalescent plasma. However, neutralizing antibody responses or RBD IgG responses were not tested in donor plasma. It is also unclear whether the timing of plasma therapy coincided with presence of the virus – both which has been demonstrated to be critical to reap clinical benefits of convalescent plasma therapy (Donato et al., 2021; Ray et al., 2020).

Our study raises important questions on formation of protective immune memory after recovering from COVID-19. Consistent with a previous study (Kalkan Yazici et al., 2020), we found that nearly half of the COVID-19 recovered individuals did not induce 50% neutralizing titers even at 1:20 dilution of plasma. This raises the question of whether these individuals with low neutralizing antibodies also differ in formation of cellular immune memory. Our data show that individuals with low neutralizing antibodies indeed had lower memory B cells. Given that T cells may also contribute to COVID-19 protection, studies are needed to understand whether these individuals may also differ in the generation of memory CD8 and CD4 T cells (Chen and John Wherry, 2020;

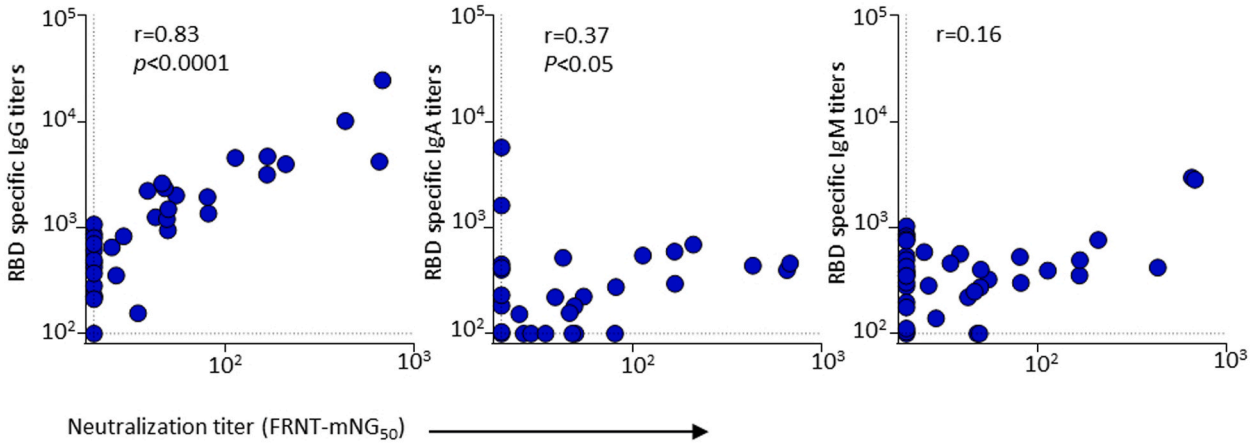

Fig. 4. ***Correlation analysis of SARS-CoV-2-specific antibody responses versus neutralization titers.*** Correlation analysis shows FRNT-mNG$_{50}$ titers (x-axis) versus RBD-specific IgG (Left), IgA (middle) and IgM (right) titers on y-axis in COVID-19 recovered individuals ($n = 42$, blue dots). Correlation analysis was performed by log transformation of the endpoint ELISA titers followed by linear regression analysis. Dotted line on x-axis and y-axis indicate limit of detection. Correlations were calculated by Spearman's correlation coefficient r. $p \leq 0.05$ was considered significant and indicated in the figure.

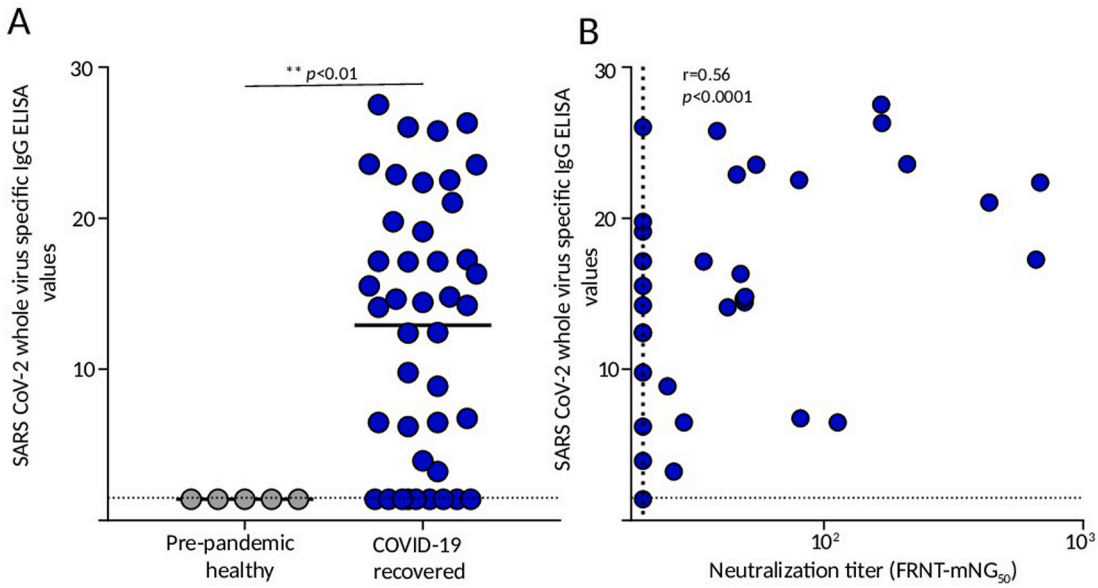

Fig. 5. Correlation analysis of SARS-CoV-2 whole virus specific IgG versus neutralizing titers. (A). Scatter plots shows SARS-CoV-2 whole virus specific IgG measured using measured using commercial kit (Zydus diagnosis, Covid Kavach) in pre-pandemic healthy (n = 5) and COVID-19 recovered (n = 42). The unpaired analysis was done using non-parametric Mann-Whitney-U test. $p \leq 0.05$ was considered significant. **(B).** Correlation analysis of SARS-CoV-2 whole virus antigen specific IgG ELISA kit values (y-axis) versus neutralizing titers (x-axis) in COVID-19 recovered individuals (n = 42). Correlations were calculated by Spearman's correlation coefficient r. $p \leq 0.05$ was considered significant. Dotted line on x-axis indicate limit of detection and on y-axis assay cut off.

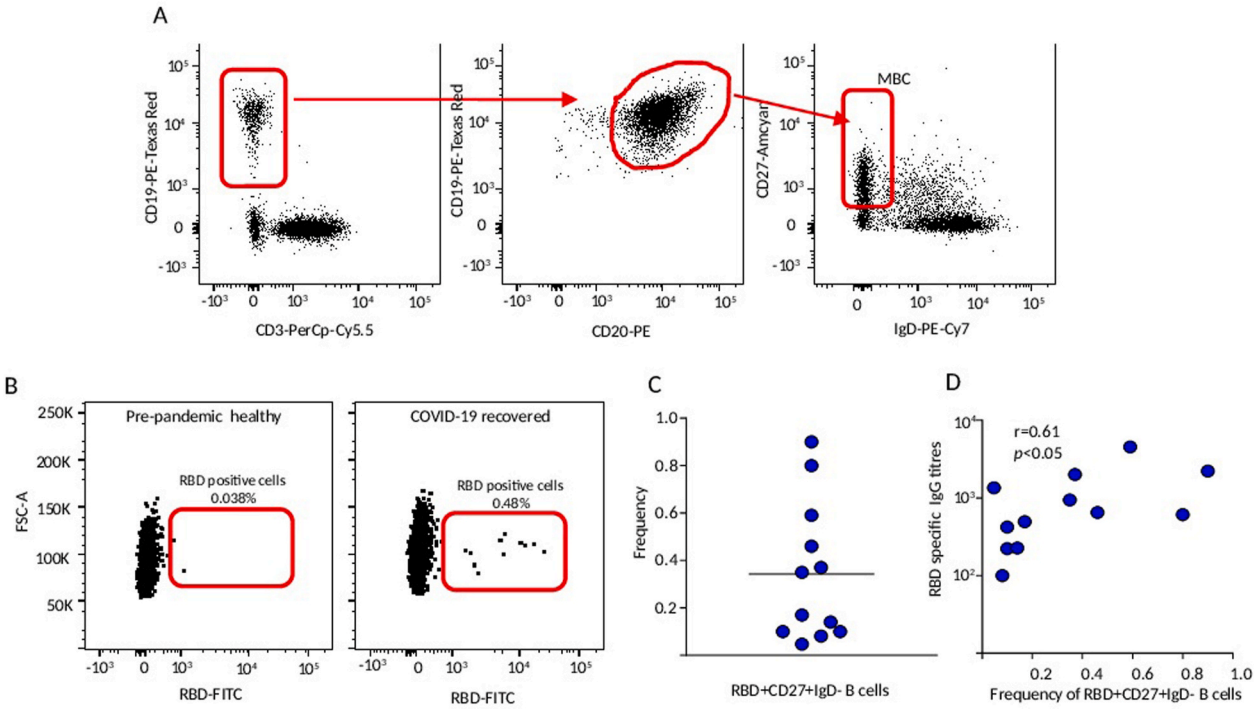

Fig. 6. SARS-CoV-2 RBD-specific memory B cell analysis in COVID-19 recovered individuals. (A) Gating strategy used to identify memory B cells. **(B)** SARS-CoV-2 RBD-specific memory B cells on gated total memory B cells that were CD19 positive, CD20 high, IgD negative and CD27 high is shown. **(C)** Frequency of RBD-specific memory B cells of the total memory B cells in the COVID-19 recovered individuals (n = 13). **(D)** Correlation analysis shows frequency of RBD-specific memory B cells (x-axis) and the RBD-specific IgG titers (y-axis) in COVID-19 recovered individuals.

Grifoni et al., 2020; Jesenak et al., 2020).

The reason why only half of the COVID-19 recovered individuals developed appreciable levels of neutralizing antibody titers requires further investigation. This may be related to inter-individual differences in human immune responses associated with the expected heterogeneity in initial viral inoculum (Welten et al., 2016), initial viral loads (Akondy et al., 2015; Arankalle et al., 2010; Reddy et al., 2014), incubation period (Hermesh et al., 2010), host genetic factors (Carter-Timofte et al., 2020; Hou et al., 2020; LoPresti et al., 2020) and disease severity (Seow et al., 2020; Kong et al., 2020; Liu et al., 2020b). This is consistent with previous studies that show relatively higher neutralizing antibodies in COVID-19 hospitalized patients during the acute febrile phase, or in

recovered individuals that were previously hospitalized with severe COVID-19 disease (Kong et al., 2020; Liu et al., 2020b). It is noteworthy that the COVID-19 recovered individuals from our study had mild to moderate symptoms during the initial diagnosis and thus our study cannot substantiate whether a higher proportion of individuals may have shown appreciable neutralizing titers if they had more severe symptoms during the acute stage. In light of these studies, our findings warrant future studies to seek an understanding of whether the individuals that have generated low or no neutralizing antibodies, IgG titers or memory B cells past recovery will be protected if they were re-exposed to SARS-CoV-2 or a related virus.

Author contributions

Experimental work, data acquisition and analysis of data by K.N, K. G, S.K, E.S.R, V.V.E., K.F, R.K, S.L. C. D, J.W, M.S.S, and D.S. Clinical site coordination by D. S, P.K.G, S.A, A.S and M.R. Conceptualization and implementation by A.S, R.A, K.M, A.C. Manuscript writing by A.C and K. M. All authors contributed reviewing and editing the manuscript.

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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