SARS-CoV-2 Omicron triggers cross-reactive neutralization and Fc effector functions in previously vaccinated, but not unvaccinated individuals

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Summary

The SARS-CoV-2 Omicron variant largely escapes neutralizing antibodies elicited by vaccines or infection. However, whether Omicron triggers humoral responses that are cross-reactive to other variants of concern (VOCs) remains largely unknown. We use plasma from 20 unvaccinated and seven vaccinated individuals infected during the Omicron wave in South Africa to test binding, antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and neutralization against VOCs. In unvaccinated individuals, Fc effector function and binding antibodies target Omicron and other VOCs at comparable levels. However, Omicron-triggered neutralization is not extensively cross-reactive to VOCs, with 20 to 43-fold reductions in titer. In contrast, vaccination followed by breakthrough Omicron infection improved cross-neutralization of VOCs, with titers exceeding 1:2,900. This has important implications for the vulnerability of unvaccinated Omicron-infected individuals to reinfection by circulating and emerging VOCs. Further, while Omicron-based immunogens may be adequate boosters, they are unlikely to be superior to existing vaccines for priming in SARS-CoV-2 naïve individuals.

The emergence of the SARS-CoV-2 Omicron (B.1.1.529) variant of concern (VOC) in November 2021 coincided with the fourth wave of South Africa's COVID-19 epidemic[1].

Omicron is defined by multiple mutations across its genome, including more than 30 mutations in the spike protein, many of which are associated with immune evasion[1]. These mutations confer neutralization escape across multiple vaccine platforms and in donors previously infected with other variants[2–5].

In contrast to neutralization, antibody binding to the Omicron variant is preserved, as previously observed for other VOCs[6,7]. Although Fc receptor binding has been shown to be substantially reduced against Omicron[8], functional Fc effector responses have not yet been reported. The ability of antibodies to bind the Omicron spike suggests that cytotoxic effector functions driven by antibodies may also be retained, as for earlier VOCs[9,10]. This, along with the finding that T cells triggered by either infection or vaccination are cross-reactive for Omicron[11–13], likely contributes to maintained vaccine effectiveness against severe disease following Omicron infection[14].

While there is substantial data showing that Omicron evades neutralizing immune responses, little is known about the humoral response that Omicron infection itself triggers. Defining the capacity of Omicron to trigger cross-reactive binding, neutralizing and Fc effector responses antibodies will inform its potential to protect from reinfection by currently circulating or emerging VOCs. This is of particular relevance for developing countries such as South Africa, where vaccination levels are low and genomic surveillance indicates continued circulation of Delta and potentially C.1.2 and Beta which were still circulating in December 2021. Secondly, these data inform the potential immunogenicity of Omicron-based vaccines which are under development by several companies.

Plasma from individuals infected in the fourth wave of COVID-19 pandemic in South Africa were used to assess cross-reactivity against different VOCs for binding, Fc effector function and neutralization. Plasma was used from 27 hospitalized individuals from Tshwane District Hospital recruited between 25 November 2021 and 20 December 2021 when Omicron was

responsible for >90% of infections[1] (Table S1). Of these, seven plasma samples had matched nasal swabs available and all were confirmed as Omicron BA.1 infections by sequencing. Twenty individuals were unvaccinated with no history of previous symptomatic COVID-19 infection. Seven individuals had previously been vaccinated with either one dose of Ad26.CoV2.S (n=2) or two doses of BNT162b2 (n=5) at least 56 days (56-163 days) prior to infection. Samples were taken a median of four days (1-10 days) after a positive PCR test. The median ages of the vaccinated individuals were similar to those of the unvaccinated group (58 and 64 respectively), and infections ranged from mild to severe as determined by WHO scoring (Table S1).

We first compared binding antibody levels, as measured by enzyme-linked immunosorbent assay (ELISA) against the ancestral D614G, Beta, Delta and Omicron spikes. In unvaccinated individuals, binding antibody titers against Omicron were highest, as expected, and were detectable in all donors. Although we observed statistically significant 2.2, 1.8 and 1.7-fold decreases in binding to D614G, Beta and Delta respectively in this group, Omicron-triggered antibodies were fairly cross-reactive for all variants tested, losing activity against other VOCs in 10-25% of individuals (Figure 1A, C). In previously vaccinated individuals who experienced breakthrough infection with Omicron, binding was substantially higher against Omicron than in unvaccinated individuals (geometric mean titer (GMT) of 2.92 versus 1.92) (Figure 1B, C). Furthermore, antibodies from these vaccinated individuals exhibited higher levels of cross-reactivity against all variants, and no significant fold losses were observed (Figure 1B).

As spike binding antibodies perform Fc effector functions known to contribute to reduced disease severity and vaccine efficacy[15,16], we examined antibody dependent cellular phagocytosis (ADCP) and antibody dependent cellular cytotoxicity (ADCC) in both groups. For unvaccinated individuals, ADCP against Omicron was detected in all 20 individuals with a geometric mean (GM) score of 32 (Figure 1D). Against VOCs, we observed less than two-fold reduction in activity across variants with 15%, 25% and 10% of individuals losing ADCP activity

against D614G, and Beta and Delta respectively. For vaccinated individuals, non-significant reductions against D614G, Beta and Delta were observed relative to Omicron and all donors exhibited activity against the panel of VOC tested here (Figure 1E). Compared to unvaccinated individuals, significantly higher levels of ADCP were observed in vaccinated individuals infected with Omicron, mirroring the binding antibodies (Figure 1E, F).

In contrast to binding and ADCP, ADCC in unvaccinated individuals showed significant losses against D614G (3-fold loss) and Beta (4-fold loss). However, like ADCP and binding antibodies, ADCC activity against Delta was retained (Figure 1G). In this group, Omicron-triggered ADCC was undetectable against D614G and Beta in 25% and 30% of plasma samples, respectively. After previous vaccination, Omicron breakthrough infections resulted in overall preservation of ADCC against VOCs, with only one individual showing undetectable activity against Delta (Figure 1H). Levels of ADCC in previously vaccinated donors were significantly higher than those in unvaccinated individuals, with the exception of Delta, where similar ADCC activity was observed in both groups (Figure 1I).

Lastly, we measured neutralizing antibody responses to Omicron, and assessed their cross-reactivity for VOCs. In addition to those variants tested for Fc effector function, we also tested C.1.2, a variant with several neutralization evasive mutations, which circulated at low levels during the third and fourth COVID-19 waves in South Africa[17]. Against Omicron, unvaccinated individuals showed potent neutralization, with a GMT of 4,288. However, against VOCs, we saw dramatic reductions of 27-, 43-, 23- and 20-fold in titer for D614G, Beta, C.1.2 and Delta, respectively, and knock out ranging from 27% to 45% of plasma tested (Figure 2A), particularly evident for Beta. This loss of neutralization was mitigated by prior vaccination, with all seven breakthrough infections resulting in a significantly increased GMT of 12,197 against Omicron, and high titers against all VOCs (25,263 for D614G; 2,913 for Beta; 4,506 against C.1.2 and 3004 against Delta) (Figure 2B, C). These greatly enhanced titers resulted in far greater fold increases between previously vaccinated individuals and unvaccinated individuals (158, 29,

14 and 3-fold for D614G, Beta, Delta and Omicron respectively) compared to those seen for Fc effector functions and binding which ranged from 1 to 3 fold (Figure 1 C, F, I). Notably, Omicron infection elicited robust and similar neutralization titers against itself regardless of vaccination status.

While the neutralization resistance of Omicron is now well-defined, here we address the question of how effectively Omicron-elicited antibodies target D614G and other VOCs. We show that in previously unvaccinated individuals, Omicron-triggered antibodies bind and perform Fc effector function with only slight loss against VOCs. However, neutralization was significantly compromised against VOCs, indicating limited neutralization cross-reactivity of antibodies elicited by Omicron. In contrast, vaccinated individuals who subsequently became infected with Omicron, showed greatly improved cross-reactivity with high titers against Omicron, D614G (one amino acid different from the vaccine spike), Beta, Delta and C.1.2.

We and others have previously shown that Fc effector function is largely preserved against VOCs in both convalescent and vaccine-elicited plasma[9,10]. Also, as with neutralization, we have shown that Fc effector function triggered by Beta is substantially more cross-reactive compared to antibodies elicited by D614G, indicating that the spike sequence of the eliciting immunogen impacts the extent of ADCC cross-reactivity [9,18]. Here, we show that Omicron infection similarly triggers differential ADCC cross-reactivity, with significantly decreased activity against D614G and Beta, but not Delta. This observation extended to vaccinated individuals, where even boosted ADCC was still significantly poorer against Beta. This differential targeting of ADCC-mediating antibodies indicates that they may preferentially bind sites that differ between Omicron and other VOCs. Alternatively, different VOCs may trigger antibodies with varied glycosylations and isotype, both of which are known to modulate Fc effector function[19].

This differential immune imprinting by VOCs was also confirmed for neutralization in this study in both unvaccinated and vaccinated Omicron-infected individuals. The highest titers in unvaccinated individuals were to the infection-matched Omicron. In contrast, in previously vaccinated individuals with breakthrough Omicron infections, high titers were observed against both D614G and Omicron; sequences that match the vaccine and infecting spikes to which these donors have been exposed. This is consistent with our previous studies indicating that the sequence of the infecting spike impacts the quality of the neutralization, suggesting imprinting of the immune response[20].

We and others have shown that humoral function is significantly boosted in individuals with breakthrough infections after vaccination[21–23]. This study confirms that this is also true of Omicron breakthrough infections, with a 153-fold increase in titers to D614G in vaccinated compared to unvaccinated individuals, consistent with other studies[23–25]. We also extend our previous study, where we showed ADCC was boosted by breakthrough infection, to include ADCP, confirming that this applies to other Fc effector functions[21].

In the absence of vaccination, Omicron-elicited humoral responses, while potent against the matched Omicron spike, show significantly less activity against VOCs. Thus, while highly immunogenic, Omicron does not elicit cross-neutralizing responses. This is consistent with a decrease in the ability of plasma from unvaccinated individuals to neutralize Delta compared to Omicron following Omicron infection[25]. This may result in risk of reinfection in this unvaccinated group with other variants that continued to circulate and evolve in South Africa at the time of this study, albeit at low levels including Beta, Delta and C.1.2.

Our data also have implications for the design of second-generation vaccines based on Omicron, suggesting that this may not trigger cross-reactive *de novo* responses in SARS-CoV-2 naive individuals. This is supported by immunogenicity studies, where Delta-infected mice elicited broadly protective antibodies, but Omicron-infected mice failed to mount responses

against other VOCs[23]. In addition, immunization of mice with a receptor binding domain (RBD)-based Omicron mRNA vaccine only elicited strain-specific neutralization[26]. In support of this study and others[27], our data also suggests that Omicron is highly immunogenic, eliciting comparable neutralization titers irrespective of vaccination status but only in terms of a strain-specific response. Given the significant boost we see against Omicron in vaccinated individuals, Omicron boosters may be effective in seropositive individuals, a group that exceeds 70% in South Africa[28]. However, in a comparison of mRNA-1273 vaccinated rhesus macaques boosted with either mRNA-Omicron or mRNA-1273, Omicron boosted animals showed lower titers than those with a homologous mRNA-1273 boost[29]. Overall, these data suggest that boosting individuals with or without immunity with vaccines specific for Omicron is unlikely to be superior to existing regimens.

Our study is limited by the fact that we cannot rule out prior asymptomatic infection, and that viral sequences confirming Omicron infection are available only for a subset of samples. However, we note that Omicron overwhelmingly dominated infections during the wave in which these individuals were tested[1]. Further, the median age of individuals in this study is advanced (58 years) and may contribute to comprised cross-reactivity noted for unvaccinated individuals. Given the high prevalence of global seropositivity, through vaccination or previous infection, the ability to measure the response to Omicron infection in naive samples is limited. As such our study offers valuable insights into the usefulness of Omicron as an immunogen, the potential risk of reinfection in unvaccinated individuals, and the observation that cross-reactive responses are greatly improved by vaccination.

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Author contributions

S.I.R designed the study, performed experiments, analyzed the data and wrote the manuscript. N.P.M and H.S performed Fc experiments and analyzed data. V.S.M, N.M and T.M performed neutralization assays. B.E.L, B.O. and F.A. produced spikes and variant plasmids. F.A and Z.M performed ELISA assays supervised by T.M.G. M.A. vdM processed samples which were recruited by Z. dB., T.R d. V., A.B. and G.vdB. V.U, T.R. and M.T.B established the Pretoria COVID-19 study which provided participant samples from the Tshwane District Hospital. A.S, A.M, H.T., M.V., and TdO performed SARS-CoV-2 sequencing. PLM conceptualized the study and wrote the manuscript.

Declaration of Interests

All authors declare no competing interests.

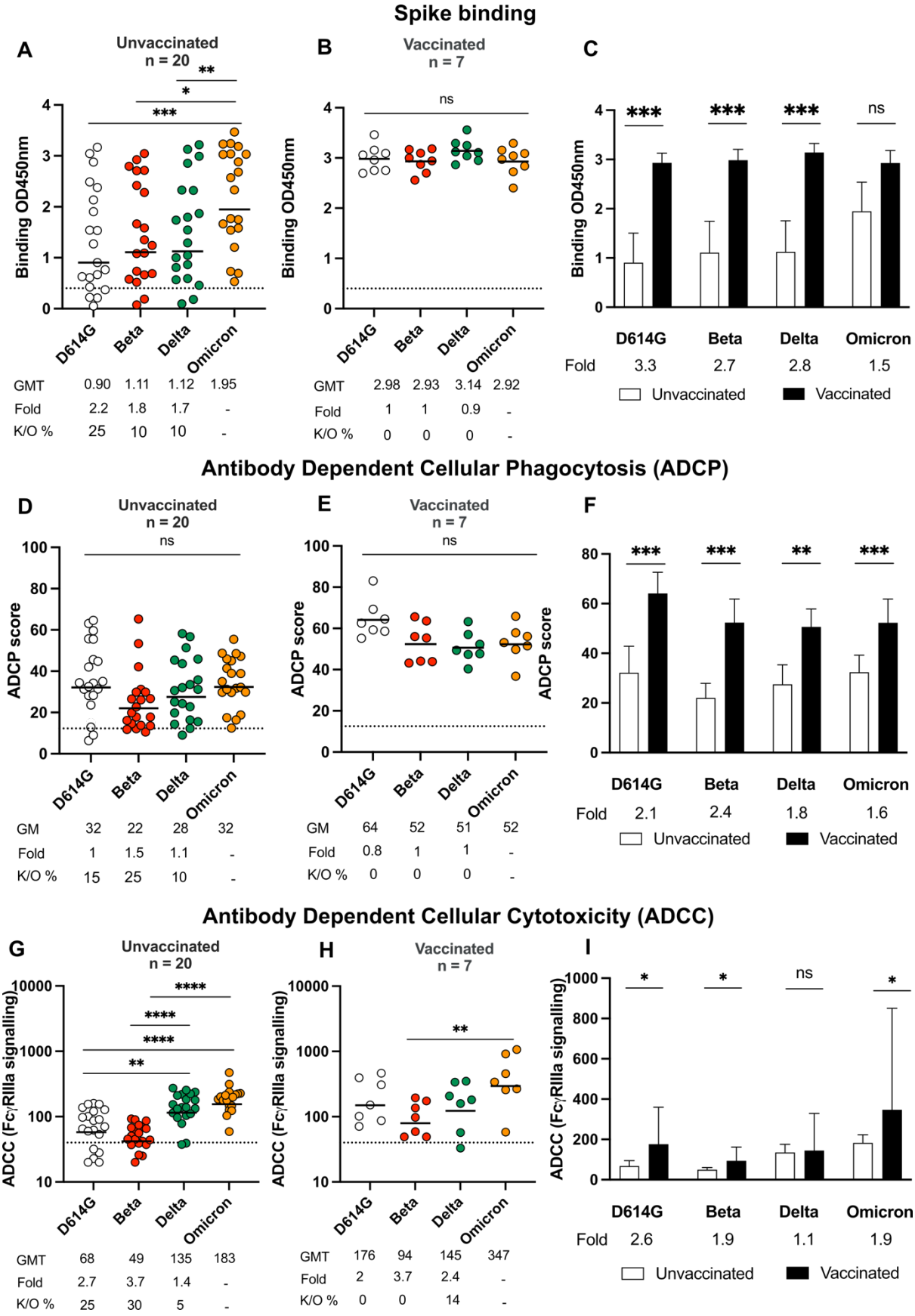

Figure 1: Binding and Fc effector function elicited by Omicron infection is cross-reactive against several variants of concern

Antibody binding measured by ELISA in (A) unvaccinated individuals (n =20) or (B) individuals vaccinated with either one dose of Ad26.CoV.2S or two doses of BNT162b2 (n=7) and infected by Omicron against D614G, Beta, Delta and Omicron spike proteins. (C) Bars show geometric mean binding titers for vaccinated (black) and unvaccinated (white) individuals against variants of concern. Antibody-dependent cellular phagocytosis (ADCP) of (D) unvaccinated and (E) vaccinated individuals is represented as the percentage of monocytic cells that take up spike coated beads (D614G, Beta, Delta and Omicron) multiplied by their geometric mean fluorescence intensity (MFI). (F) Bars show geometric mean ADCP scores for vaccinated (black) and unvaccinated (white) individuals against variants of concern. Antibody dependent cellular cytotoxicity (ADCC) in (G) unvaccinated and (H) vaccinated individuals shown as relative light units (RLU) signaling through FcγRIIIa expressing cells. (I) Bars show geometric mean activity for vaccinated (black) and unvaccinated (white) individuals against variants of concern. All data are representative of two independent experiments. For dot plots, lines indicate geometric mean titer (GMT) also represented below the plot with fold decrease and knock-out (K/O) of activity for other variants as a percentage relative to Omicron. Dotted lines indicate the limit of detection of the particular assay. For bar charts, bars indicate median of function, with error bars showing standard deviations with fold decreases relative to vaccinated individuals indicated below the plot. Statistical significance across variants is shown by Friedman test with Dunn's correction and between vaccinated and unvaccinated samples by the Mann Whitney test. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 and ns = non-significant.

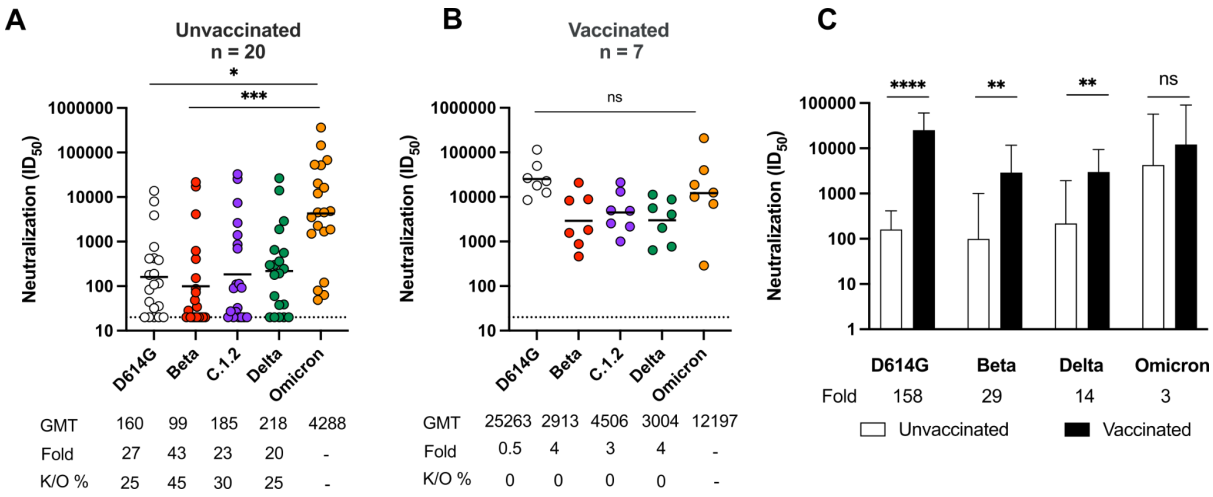

Figure 2: Omicron triggers cross-variant neutralizing antibodies which are broadened by vaccination

Neutralization titer (ID_{50}) of Omicron-infected plasma against D614G, Beta, C.1.2, Delta and Omicron pseudoviruses shown for (A) unvaccinated individuals (n=20) or (B) individuals vaccinated with either one dose of Ad26.CoV.2S or two doses of BNT162b2 (n=7). Lines indicate geometric mean titer (GMT) also represented below the plot with fold decrease and knock-out (K/O) of activity for other variants as a percentage relative to Omicron. Dotted lines indicate the limit of detection of the assay. Statistical significance across variants is shown by Friedman test with Dunns correction. (C) Bars show geometric mean neutralization titers for vaccinated (black) and unvaccinated (white) individuals against variants of concern with error bars showing standard deviations with fold decreases relative to vaccinated individuals indicated below the plot. Statistical significance between vaccinated and unvaccinated samples by the Mann Whitney test. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 and ns = non-significant. All data are representative of two independent experiments.

Table S1: Demographic and clinical description of the cohort

Study number	Age	Gender	Days sampled post infection	Vaccination status	Days since vaccination	Sequence	Severity
COV132	36-40	Male	9	Unvaccinated	-	Not sequenced	Severe
COV140	26-30	Female	1	Unvaccinated	-	Not sequenced	Mild
COV145	16-20	Female	3	Unvaccinated	-	Omicron	Mild
COV149	60-65	Female	3	Unvaccinated	-	Not sequenced	Mild
COV157	66-70	Male	4	Unvaccinated	-	Omicron	Moderate
COV158	66-70	Female	6	Unvaccinated	-	Not sequenced	Moderate
COV159	20-25	Male	6	Unvaccinated	-	Not sequenced	Mild
COV162	56-60	Male	5	Unvaccinated	-	Not sequenced	Moderate
COV165	60-65	Female	4	Unvaccinated	-	Not sequenced	Moderate
COV166	56-60	Male	4	Unvaccinated	-	Not sequenced	Severe
COV167	60-65	Female	8	Unvaccinated	-	Not sequenced	Severe
COV176	70-75	Male	2	Unvaccinated	-	Not sequenced	Moderate
COV178	60-65	Female	2	Unvaccinated	-	Omicron	Moderate
COV183	26-30	Male	2	Unvaccinated	-	Not sequenced	Mild
COV184	56-60	Male	1	Unvaccinated	-	Not sequenced	Moderate
COV185	46-50	Female	1	Unvaccinated	-	Not sequenced	Moderate
COV186	56-60	Male	10	Unvaccinated	-	Omicron	Moderate
COV187	30-35	Female	3	Unvaccinated	-	Not sequenced	Mild
COV189	46-50	Male	5	Unvaccinated	-	Not sequenced	Moderate
COV190	30-35	Female	4	Unvaccinated	-	Omicron	Mild
COV151	46-50	Male	1	One dose Ad26.CoV2.S	56	Omicron	Severe
COV182	36-40	Male	3	One dose Ad26.CoV2.S	Unknown	Not sequenced	Mild
COV131	56-60	Female	4	Two doses BNT162b2	109	Not sequenced	Severe
COV135	80-85	Male	5	Two doses BNT162b2	72	Omicron	Mild
COV138	66-70	Male	5	Two doses BNT162b2	Unknown	Not sequenced	Moderate
COV161	60-65	Female	5	Two doses BNT162b2	112	Not sequenced	Mild
COV181	70-75	Male	4	Two doses BNT162b2	163	Not sequenced	Severe

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Methods

RESOURCE AVAILABILITY

Lead Contact

Further information and reasonable requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Penny Moore (pennym@nicd.ac.za).

Materials availability

Materials will be made by request to Penny Moore (pennym@nicd.ac.za).

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the Lead Contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Human Subjects

Samples infected in the fourth COVID-19 wave of infection in South Africa were collected from participants enrolled to the Pretoria COVID-19 study cohort. Participants were admitted to Tshwane District Hospital (Pretoria, South Africa) with mild to severe PCR confirmed SARS-CoV-2 infection between 25 November 2021- 20 December 2021 (Table S1). Ethics approval was received from the University of Pretoria, Human Research Ethics Committee (Medical) (247/2020). All patients had PCR confirmed SARS-CoV-2 infection before blood collection which was done a median of 4 days post positive PCR test. Written informed consent was obtained from all participants.

Cell lines

Human embryo kidney HEK293T cells were cultured at 37°C, 5% CO2, in DMEM containing 10% heat-inactivated fetal bovine serum (Gibco BRL Life Technologies) and supplemented with 50 µg/ml gentamicin (Sigma). Cells were disrupted at confluence with 0.25% trypsin in 1 mM EDTA (Sigma) every 48–72 hours. HEK293T/ACE2.MF cells were maintained in the same way as HEK293T cells but were supplemented with 3 µg/ml puromycin for selection of stably transduced cells. HEK293F suspension cells were cultured in 293 Freestyle media (Gibco BRL Life Technologies) and cultured in a shaking incubator at 37°C, 5% CO2, 70% humidity at 125rpm maintained between 0.2 and 0.5 million cells/ml. Jurkat-Lucia™ NFAT-CD16 cells

were maintained in IMDM media with 10% heat-inactivated fetal bovine serum (Gibco, Gaithersburg, MD), 1% Penicillin Streptomycin (Gibco, Gaithersburg, MD) and 10 µg/ml of Blasticidin and 100 µg/ml of Zeocin was added to the growth medium every other passage. THP-1 cells were used for both the ADCP and ADCT assays and obtained from the AIDS Reagent Program, Division of AIDS, NIAID, NIH contributed by Dr. Li Wu and Vineet N. KewalRamani. Cells were cultured at 37°C, 5% CO2 in RPMI containing 10% heat-inactivated fetal bovine serum (Gibco, Gaithersburg, MD) with 1% Penicillin Streptomycin (Gibco, Gaithersburg, MD) and 2-mercaptoethanol to a final concentration of 0.05 mM and not allowed to exceed 4 x 10^5 cells/ml to prevent differentiation.

METHOD DETAILS

SARS-CoV-2 spike genome sequencing

Sequencing of the spike was performed as previously described[30] using swabs obtained from Tshwane District Hospital patients of which 6 were available and confirmed to be Omicron (Table S1). RNA sequencing was performed as previously published. Briefly, extracted RNA was used to synthesize cDNA using the Superscript IV First Strand synthesis system (Life Technologies, Carlsbad, CA) and random hexamer primers. SARS-CoV-2 whole genome amplification was performed by multiplex PCR using primers designed on Primal Scheme (http://primal.zibraproject.org/) to generate 400 bp amplicons with a 70 bp overlap covering the SARS-CoV-2 genome. Phylogenetic clade classification of the genomes in this study consisted of analyzing them against a global reference dataset using a custom pipeline based on a local version of NextStrain (https://github.com/nextstrain/ncov)[31].

SARS-CoV-2 antigens

For ELISA and ADCP assays, SARS-CoV-2 original and Beta variant full spike (L18F, D80A, D215G, K417N, E484K, N501Y, D614G, A701V, 242-244 del), Delta (T19R, 156-157del, R158G, L452R, T478K, D614G, P681R and D950N) and Omicron (A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, 214EPE, G339D, S371L, S373P, S375F, K417N, N440K,

G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F) proteins were expressed in Human Embryonic Kidney (HEK) 293F suspension cells by transfecting the cells with the respective expression plasmid. After incubating for six days at 37 °C, 70% humidity and 10% CO_2, proteins were first purified using a nickel resin followed by size-exclusion chromatography. Relevant fractions were collected and frozen at -80 °C until use.

SARS-CoV-2 Spike Enzyme-linked immunosorbent assay (ELISA)

Two µg/ml of spike protein (D614G, Beta, Delta or Omicron) was used to coat 96-well, high-binding plates and incubated overnight at 4 °C. The plates were incubated in a blocking buffer consisting of 5% skimmed milk powder, 0.05% Tween 20, 1x PBS. Plasma samples were diluted to 1:100 starting dilution in a blocking buffer and added to the plates. IgG secondary antibody was diluted to 1:3000 in blocking buffer and added to the plates followed by TMB substrate (Thermofisher Scientific). Upon stopping the reaction with 1 M H_2SO_4, absorbance was measured at a 450nm wavelength. In all instances, mAbs CR3022 and BD23 were used as positive controls and Palivizumab was used as a negative control.

Spike plasmid and Lentiviral Pseudovirus Production

The SARS-CoV-2 Wuhan-1 spike, cloned into pCDNA3.1 was mutated using the QuikChange Lightning Site-Directed Mutagenesis kit (Agilent Technologies) and NEBuilder HiFi DNA Assembly Master Mix (NEB) to include D614G (original) or lineage defining mutations for Beta (L18F, D80A, D215G, 242-244del, K417N, E484K, N501Y, D614G and A701V), Delta (T19R, 156-157del, R158G, L452R, T478K, D614G, P681R and D950N), C.1.2. (P9L, P25L, C136F, Δ144, R190S, D215G, Δ242-243, Y449H, E484K, N501Y, L585F, D614G, H655Y, N679K, T716I, T859N) or Omicron (A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, 214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F).

Pseudotyped lentiviruses were prepared by co-transfecting HEK293T cell line with the SARS-CoV-2 ancestral variant spike (D614G), Beta, Delta, C.1.2 or Omicron spike plasmids in conjunction with a firefly luciferase encoding lentivirus backbone plasmid as previously described[7]. Briefly, pseudoviruses were produced by co-transfection in 293T/17 cells with a lentiviral backbone (HIV-1 pNL4.luc encoding the firefly luciferase gene) and either of the SARS-CoV-2 spike plasmids with PEIMAX (Polysciences). Culture supernatants were clarified of cells by a 0.45-μM filter and stored at −70 °C. Other pcDNA plasmids were used for the ADCC assay.

Pseudovirus neutralization assay

For the neutralization assay, plasma samples were heat-inactivated and clarified by centrifugation. Heat-inactivated plasma samples from vaccine recipients were incubated with the SARS-CoV-2 pseudotyped virus for 1 hour at 37°C, 5% CO2. Subsequently, $1x10^4$ HEK293T cells engineered to over-express ACE-2 (293T/ACE2.MF)(kindly provided by M. Farzan (Scripps Research)) were added and incubated at 37°C, 5% CO_2 for 72 hours upon which the luminescence of the luciferase gene was measured. Titers were calculated as the reciprocal plasma dilution (ID_{50}) causing 50% reduction of relative light units. CB6 and CA1 was used as positive controls for D614G, Beta and Delta. 084-7D, a mAb targeting K417N was used as a positive control for Omicron and Beta.

Antibody-dependent cellular phagocytosis (ADCP) assay

Avitagged SARS-CoV-2 spikes were biotinylated using the BirA biotin-protein ligase standard reaction kit (Avidity, LLC) and coated onto fluorescent neutravidin beads as previously described[32]. Briefly, beads were incubated for two hours with monoclonal antibodies at a starting concentration of 20 μg/ml or plasma at a single 1 in 100 dilution. Opsonized beads were incubated with the monocytic THP-1 cell line overnight, fixed and interrogated on the

FACSAria II. Phagocytosis score was calculated as the percentage of THP-1 cells that engulfed fluorescent beads multiplied by the geometric mean fluorescence intensity of the population less the no antibody control. For this and all subsequent Fc effector assays, pooled plasma from 5 PCR-confirmed SARS-CoV-2 infected individuals and CR3022 were used as positive controls and plasma from 5 pre-pandemic healthy controls and Palivizumab were used as negative controls. In addition samples both waves were run head-to-head in the same experiment. ADCP scores for different spikes were normalised to each other and between runs using CR3022.

Antibody-dependent cellular cytotoxicity (ADCC) assay

The ability of plasma antibodies to cross-link and signal through FcγRIIIa (CD16) and spike expressing cells or SARS-CoV-2 protein was measured as a proxy for ADCC. For spike assays, HEK293T cells were transfected with 5µg of SARS-CoV-2 spike plasmids using PEI-MAX 40,000 (Polysciences) and incubated for 2 days at 37°C. Expression of spike was confirmed by differential binding of CR3022 and P2B-2F6 and their detection by anti-IgG APC staining measured by flow cytometry. Subsequently, $1x10^5$ spike transfected cells per well were incubated with heat inactivated plasma (1:100 final dilution) or monoclonal antibodies (final concentration of 100 µg/ml) in RPMI 1640 media supplemented with 10% FBS 1% Pen/Strep (Gibco, Gaithersburg, MD) for 1 hour at 37°C. Jurkat-Lucia™ NFAT-CD16 cells (Invivogen) ($2x10^5$ cells/well and $1x10^5$ cells/well for spike and other protein respectively) were added and incubated for 24 hours at 37°C, 5% CO_2. Twenty µl of supernatant was then transferred to a white 96-well plate with 50 µl of reconstituted QUANTI-Luc secreted luciferase and read immediately on a Victor 3 luminometer with 1s integration time. Relative light units (RLU) of a no antibody control was subtracted as background. Palivizumab was used as a negative control, while CR3022 was used as a positive control, and P2B-2F6 to differentiate the Beta from the D614G variant. 084-7D was used as a positive control for Omicron and Beta. To induce the transgene 1x cell stimulation cocktail (Thermofisher Scientific, Oslo, Norway) and 2 µg/ml ionomycin in R10 was added as a positive control to confirm sufficient expression

of the Fc receptor. RLUs for spikes were normalised to each other and between runs using CR3022. All samples were run head to head in the same experiment as were all variants tested.

QUANTIFICATION AND STATISTICAL ANALYSIS

Analyses were performed in Prism (v9; GraphPad Software Inc, San Diego, CA, USA). Non-parametric tests were used for all comparisons. The Mann-Whitney and Wilcoxon tests were used for unmatched and paired samples, respectively. The Friedman test with Dunns correction for multiple comparisons was used for matched comparisons across variants. All correlations reported are non-parametric Spearman's correlations. *P* values less than 0.05 were considered to be statistically significant.