UNIVERSITY OF KWAZULU-NATAL ™

INYUVESI YAKWAZULU-NATALI

Effect of hybrid immunity on the neutralizing antibody response to emerging SARS-CoV-2 variants including in people living with HIV

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Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy (Medical Microbiology) in the School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal

December 02, 2022

DECLARATION

I, Khadija Khan, declare as follows:

(i) The research proposed in this dissertation, except where otherwise indicated, is my original work.

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(v) Part of this thesis was published in publications, titled:

a) Immunogenicity of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-cov-2) infection and Ad26.CoV2.S Vaccination in people living with human immunodeficiency virus (HIV)
b) Omicron infection enhances antibody immunity in vaccinated persons
c) Omicron BA.4/BA.5 escape neutralizing immunity elicited by BA.1 infection

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Khadija Khan (Student)  02 December 2022

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LIST OF ACRONYMS

2019-nCoV	2019 Novel coronavirus
ACE2	Angiotensin converting enzyme-2
ART	Antiretroviral therapy
AU	African union
BTI	Breakthrough infection
CoV	Coronavirus
COVID-19	Coronavirus Disease 2019
CTs	Chest computed tomographic scans
HCoV	Human coronavirus
HCW	Health care workers
HIV	Human Immunodeficiency Virus
ICU	Intensive care unit
LVNA	Live virus neutralization assay
mAbs	Monoclonal antibodies
MERS-CoV	Middle East respiratory syndrome coronavirus
mRNA	Messenger ribosomal nucleic acid
nAb	Neutralizing antibodies
NICD	National Institute of Communicable Diseases
NTD	N-Terminal Domain
ORFs	Open reading frames
PLWH	People living with HIV
RBD	Receptor binding domain
RNA	Ribosomal nucleic acid
S	Spike

S1	Spike-1
S2	Spike-2
SA	South Africa
SARS	Severe acute respiratory syndrome
SARS-CoV	Severe acute respiratory syndrome coronavirus
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SGTF	S-gene target failure
TMPRSS2	Transmembrane protease serine 2
VoC	Variant of concern
VoI	Variant of interest
WHO	World Health Organization

ABSTRACT

This work focused on the effect of SARS-CoV-2 variants and co-infection with HIV on the neutralizing antibody response elicited by SARS-CoV-2 infection and vaccination. The first study described the effect of HIV status and suppression on antibody neutralization capacity against the Delta variant elicited by previous infection, the Janssen adeno vectored Ad26.CoV2.S vaccine, or hybrid immunity from infection followed by Ad26.CoV2.S vaccination. It was determined that while neutralizing immunity elicited by infection decreased in people living with HIV (PLWH) and particularly in those with unsuppressed HIV viremia, this effect was counteracted with hybrid immunity from infection and Ad26.CoV2.S vaccination, where with hybrid immunity neutralization levels increased and differences between PLWH and HIV negative individuals became non-significant. With the emergence of the Omicron variant, we investigated whether Omicron infection boosts cross-neutralizing antibody levels against other variants. It was determined that this cross-protection does occur but is considerably stronger in people with hybrid immunity consisting of Pfizer BNT162b2 mRNA or the Ad26.CoV2.S vaccination followed by breakthrough infection, implying that hybrid immunity from Omicron variant infection and vaccination may prevent infection with other, more pathogenic variants. Lastly, we demonstrated the escape of the BA.4 and BA.5 subvariants from Omicron BA.1 subvariant elicited immunity. It was found that BA.4 and BA.5 showed substantial escape from BA.1 elicited immunity in the absence of vaccination, but the escape was more moderate in individuals with hybrid immunity from BA.1 infection combined with vaccination. Overall, these studies show the capacity of hybrid immunity, consisting of vaccination and infection, to reduce the negative effects of HIV viremia and emerging variants on the ability of the pre-existing immunity to neutralize SARS-CoV-2.

OUTLINE OF THESIS

Since reports of the first cases of SARS-CoV-2 in December 2019, the disease rapidly spread across the globe, with cases increasing exponentially. With little information available it was important to understand viral transmissibility and disease severity of COVID-19 disease. This was accomplished by various research projects highlighted in this thesis. The thesis is organised as follows; Chapter 1 reviews the literature pertaining to the published research. Topics include SARS-CoV-2 epidemiology, variants, evolution of the virus, and immune response including vaccination against disease. The work described in Chapter 2 is published in the Journal of Clinical Infectious Diseases and presents findings on the immunogenicity of SARS-CoV-2 infection and vaccination with Ad.26.CoV2.S in PLWH. The work outlined in Chapter 3 is published in Nature and presents findings on boosting of immunity to other variants by Omicron BA.1 breakthrough infection. The work detailed in Chapter 4 is published in Nature Communications and presents findings on Omicron BA.4/BA.5 escape from neutralizing immunity elicited by BA.1 infected participants and attenuation of this escape by hybrid BA.1 infection/vaccination immunity.

CHAPTER 1: LITERATURE REVIEW

1.1. Coronaviruses

A severe respiratory disease was reported in Wuhan, Hubei province, China, with the first patient hospitalized on December 12, 2019, (Wu et al., 2020, Huang et al., 2020b). Initial investigations suggested that the outbreak was associated with a seafood market in Wuhan (Wu et al., 2020). Genome sequencing results showed the causative agent emanating from the family *Coronaviridae* with a close resemblance to a group of severe acute respiratory syndrome (SARS) like coronaviruses (genus *Betacoronavirus*, subgenus *Sarbecovirus*). This virus is the seventh member of the coronavirus family to infect humans (Huang et al., 2020b, Wu et al., 2020). Since the emergence of the Coronavirus disease (COVID-19) in China, the virus evolved and rapidly spread to other countries worldwide creating a global threat (Liu et al., 2020).

Coronaviruses, the largest group within the *Nidovirales* (Figure 1) are zoonotic viruses belonging to the *Coronaviridae* family (Masters and Perlman, 2013). Based on their genomic structure and phylogenetic relationships, the subfamily consists of four genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus* (Cui et al., 2019). The *Alphacoronaviruses* and *Betacoronaviruses* are suspected to infect only mammals, causing respiratory illness in humans and gastroenteritis in animals. The *Gammacoronaviruses* and *Deltacoronaviruses* mostly infect birds (Kirtipal et al., 2020, Cui et al., 2019, Leao et al., 2022, Masters and Perlman, 2013).

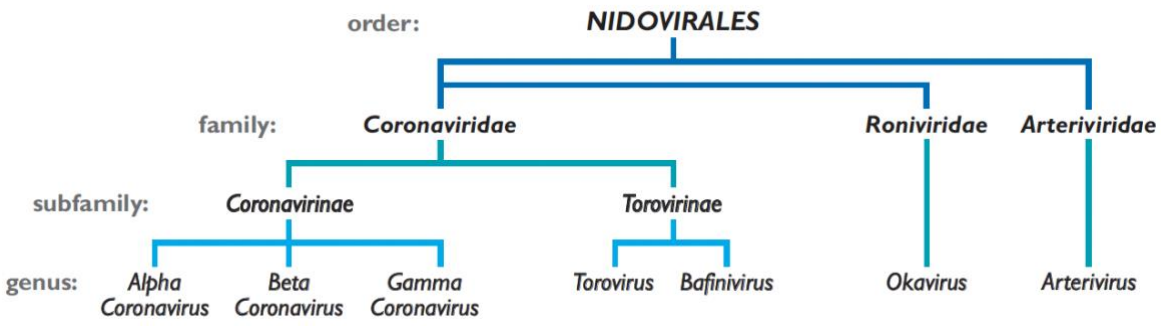

Figure 1: Taxonomy of the order *Nidovirales* (Masters and Perlman, 2013).

Coronaviruses are single-stranded, positive ribosomal nucleic acid enveloped (RNA) viruses studied for decades and known to be partially responsible for the common cold circulating in colder months, causing mild infections in immunocompetent people (Masters, 2006, Masters and Perlman, 2013).

Coronaviruses (CoVs) are known to evolve rapidly due to the RNA genome and viral recombination (Kirtipal et al., 2020, Leao et al., 2022). It is likely that novel coronaviruses may emerge periodically in humans through frequent cross-species infections and spill-over events (Woo et al., 2012).

These viruses were not considered highly pathogenic to humans until the emergence of severe acute respiratory syndrome (SARS) in March 2003, from a health care worker in Guangdong Province, China (Kirtipal et al., 2020). The outbreak affected people in approximately 30 countries and resulted in approximately 700 deaths (https://www.cdc.gov/sars/about/fs-sars.html). Almost ten years later the Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in 2012 and zoonotic transmission was linked to dromedary camels. Clinically, MERS-CoV was similar to SARS. The disease was detected in 27 countries, resulting in approximately 2500 infections and 800 deaths (Zaki et al., 2012). Both MERS-CoV and SARS are highly pathogenic in humans and cause severe respiratory distress. Both diseases were shown to transmit from animals to humans (Masters, 2006, Masters and Perlman, 2013, Ksiazek et al., 2003, Alagaili et al., 2014, Zaki et al., 2012).

The third major human CoV outbreak occurred in December 2019 in China with bats as the possible original hosts and pangolins as potential intermediate hosts. A cluster of patients with pneumonia of unknown cause was linked to a seafood market in Wuhan, China (Wu et al., 2020). It is possible that early cases of infection were unnoticed. In January 2020, the World Health Organization (WHO) temporarily named this new virus the 2019 novel coronavirus (2019-nCoV). In February 2020 the WHO declared that the 2019-nCoV epidemic constitutes a public health emergency of international concern labelling the infection as COVID-19 (Coronavirus Disease 2019), by SARS-CoV-2 and in March 2020 declared the COVID-19 outbreak a global pandemic (Leao et al., 2022, Woo et al., 2012, Kirtipal et al., 2020, Zhu et al., 2020b, Wu et al., 2020, Cucinotta and Vanelli, 2020, Ye et al., 2020).

1.2. Epidemiology of the COVID-19 pandemic

Since reports of the first cases in December 2019, the disease rapidly spread across the globe, with cases increasing exponentially. In Africa the first COVID-19 case was identified in Egypt on February 14, 2020 (https://www.afro.who.int/news/covid-19-cases-top-10-000-africa) and South Africa's National Institute of Communicable Diseases (NICD) reported the first local case on March 5, 2020, (https://www.nicd.ac.za/first-case-of-covid-19-coronavirus-reported-in-sa) almost 3 months after the first COVID-19 case was reported (Staunton et al., 2020). By the end of the first week of March 2020, 9 African countries reported over 40 index cases (Massinga Loembé et al., 2020). By May 2020, 54 of 55 African Union (AU) member States documented over 100,000 cases and 3,100 deaths. As of

November 14, 2022, the WHO reported more than 631.9 million confirmed COVID-19 cases (Figure 2) and over 6.5 million covid related deaths. By early 2022, the WHO reported approximately 1.2 million new cases every 24 hours, which has now decreased to approximately 116 000 in November 2022 .

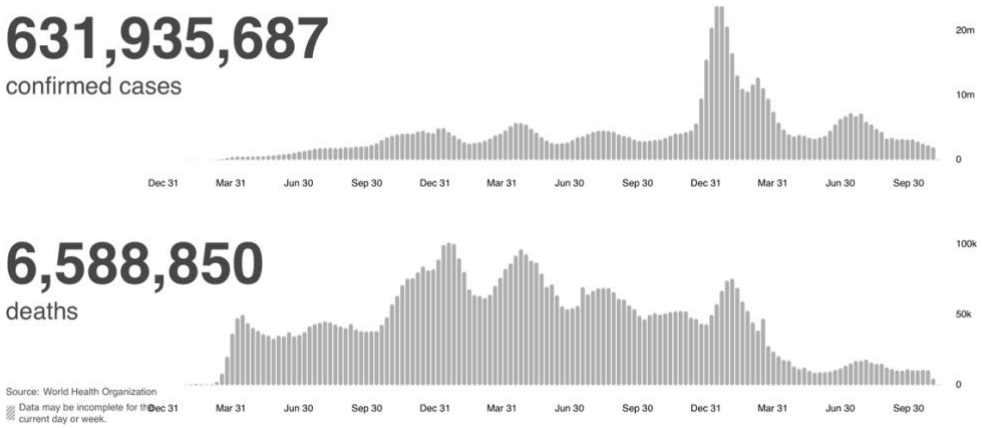

Figure 2: Number of COVID-19 cases worldwide. Source: https://covid19.who.int/.

In South Africa (SA), 21 million COVID-19 tests were performed (Figure 3) as of November 9, 2022, with the total number of confirmed cases reaching four million. The province of Gauteng experienced the highest number of COVID-19 positive cases. To date, 102,000 covid related deaths have been reported with fewer cases reported daily.

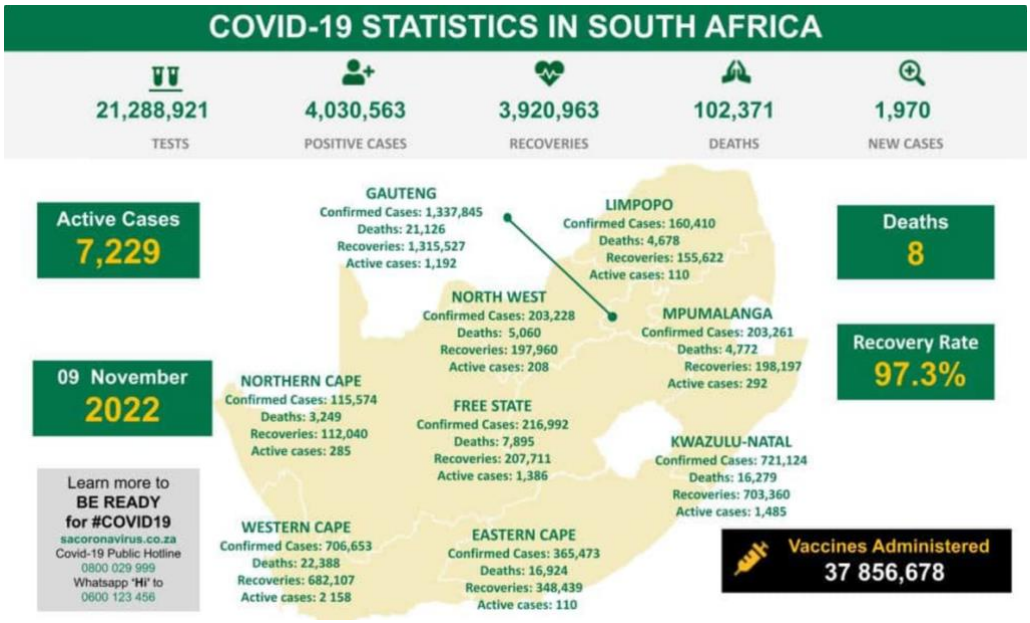

Figure 3: COVID-19 statistics in South Africa. Source: https://sacoronavirus.co.za/2022/11/11/update-on-covid-19-wednesday-09-november-2022/.

SARS-CoV-2 symptoms were similar to SARS-CoV and MERS-CoV, ranging from a dry cough, fever, dyspnoea, headache, fatigue, pneumonia, and abnormal chest computed tomographic scans (CTs), with bilateral ground-glass opacities in the lung. Complications included acute respiratory distress syndrome and associated with admissions to the intensive care unit (ICU) and high mortality (Huang et al., 2020b, Lan et al., 2020). A few patients had upper respiratory tract signs and symptoms, it was therefore thought that the target cells might be located in the lower airway (Huang et al., 2020b, Wang et al., 2020, Lan et al., 2020). Critically ill patients were older with underlying conditions such as hypertension, diabetes, cardiovascular disease, and malignancy (Harrison et al., 2020), suggesting age and comorbidity such as diabetes, hypertension, and HIV among others, may be at risk factors for poor outcomes (Wang et al., 2020, Western Cape Department of Health with the National Institute for Communicable Diseases, 2021)

1.3. Life cycle of infection

Coronaviruses are large, enveloped RNA viruses. Their genome consists of a ~30kb strand of positive sense RNA, containing 14 open reading frames (ORFs), encoding 29 viral proteins, and known to have the largest genome among all RNA viruses (Yang and Rao, 2021, Masters, 2006, Hartenian et al., 2020). The ORFs at the 3' terminus encode structural proteins; these are nucleocapsid (N) and the three membrane proteins; spike (S), membrane (M), and envelope (E) (Figure 4). These proteins are responsible for virion assembly, genome packaging, viral entry, and suppression of host immune responses (Siu et al., 2008, Hartenian et al., 2020, Yang and Rao, 2021). The N protein associates with the viral genome to direct genome packaging into new particles and participates in processes related to the response of the infected cell (Lagunas-Rangel and Chávez-Valencia, 2021). The spike (S) protein is required for viral entry, by binding to the cellular receptor and initiating fusion with the host cell membrane (Hartenian et al., 2020, Li, 2016). The M protein incorporates viral components into new virions during morphogenesis, and the E protein participates in viral assembly by an ion channel in the viral membrane, including maturation of the virus (Lagunas-Rangel and Chávez-Valencia, 2021).

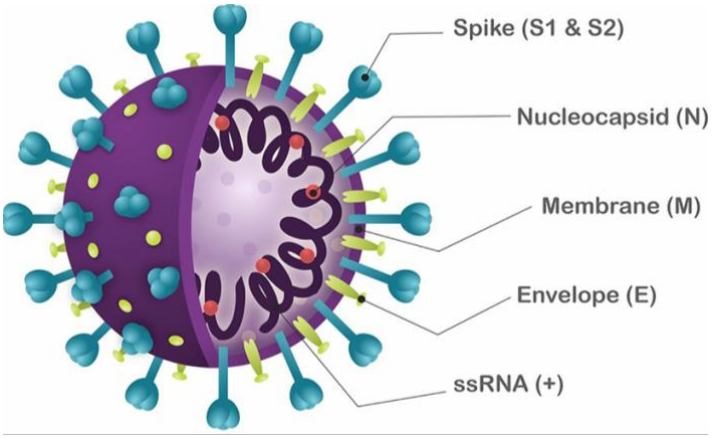

Figure 4: Schematic structure of SARS-CoV-2 (Santos et al., 2020).

The life cycle of SARS-CoV-2 involves viral entry, replication, transcription, assembly, and release. Spike mediates coronavirus entry into host cells by binding the host cellular receptor which is the human angiotensin converting enzyme-2 (ACE2) (Hoffmann et al. (2020b). It has two subunits (S1 and S2) where the S1 subunit binds the ACE2 receptor, while S2 mediates membrane fusion (Hoffmann et al., 2020b) (Figure 5). The cleavage site between S1 and S2 is predominantly cleaved by the cellular furin protease (Hoffmann et al., 2020a). This cleavage allows further cleavage at a second site (S2) mediated by the cellular serine protease TMPRSS2, activating the S2 subunit for fusion (Hoffmann et al., 2020b). The cathepsin-dependent alternate pathway for viral entry enables TMPRSS2-independent viral infection (Hoffmann et al., 2020a). The Omicron BA.1 subvariant does not have efficient S1/S2 cleavage and predominantly uses the alternative pathway to infect (Peacock et al., 2022, Willett et al., 2022, Meng et al., 2022). The receptor binding domain (RBD) of spike is the critical region which mediates attachment to ACE2 and is the primary target for neutralizing antibodies (nAb) (Huang et al., 2020c, Shang et al., 2020).

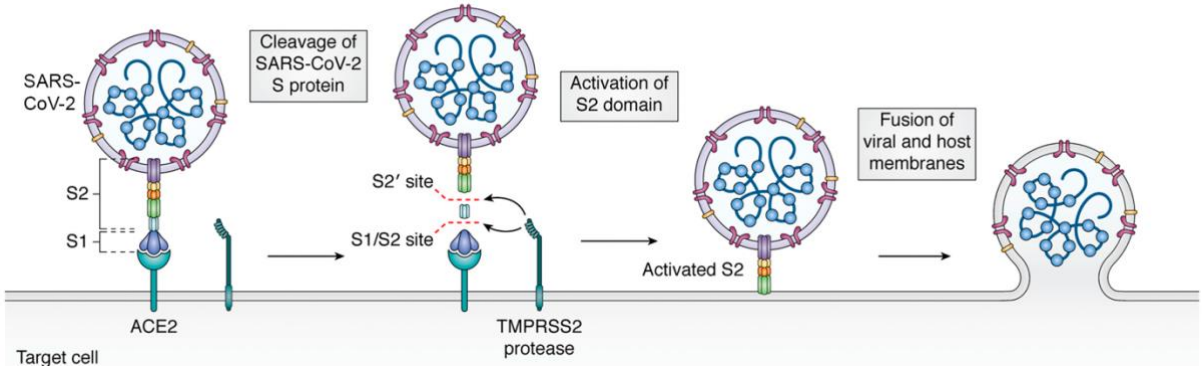

Figure 5: Mechanism of SARS-CoV-2 plasma membrane viral entry (Hartenian et al., 2020).

1.4. Evolution of SARS-CoV-2 variants

Viruses, including SARS-CoV-2, continuously evolve as changes in the genetic code occur during the replication of the genome. A single amino acid change can drastically affect the ability of the virus to evade the immune system and hamper vaccine effectiveness. At the onset of the pandemic, the genetic evolution of SARS-CoV-2 was minimal, with the ancestral SARS-CoV-2 with the D614G substitution dominating the early phase of the pandemic (Zhang et al., 2020). This substitution was identified as a missense mutation in the spike protein (Isabel et al., 2020) that had the potential to increase infectivity by assembling a more functional S protein into the virion (Zhang et al., 2020). A few months into the pandemic between October and December 2020, multiple new variants emerged (Aleem et al., 2022, Viana et al., 2021). These were labelled by the WHO as :

(a) a variant of concern (VoC); defined by an increase in transmissibility and virulence or decrease in the effectiveness of the practiced public health, social measures, and available therapeutics.

(b) a variant of interest (VoI); defined by variants observed to cause community spread to appear in multiple cases or clusters or has been detected in various countries.

(c) variant under monitoring; defined as a variant with genetic changes that are suspected to affect virus characteristics with some indication that it may pose a risk to public health and safety in the future.

Enhanced monitoring and continuous assessment is required to track the impact of variants (https://www.cdc.gov/coronavirus/2019-ncov/variants). Variants of concern pose an imminent threat as they may exhibit higher transmissibility, disease severity, or ability to evade vaccine-induced and natural immunity (Thakur et al., 2022).

South Africa experienced five periods of increased transmission of COVID-19, each fuelled by a VoC (Figure 6).

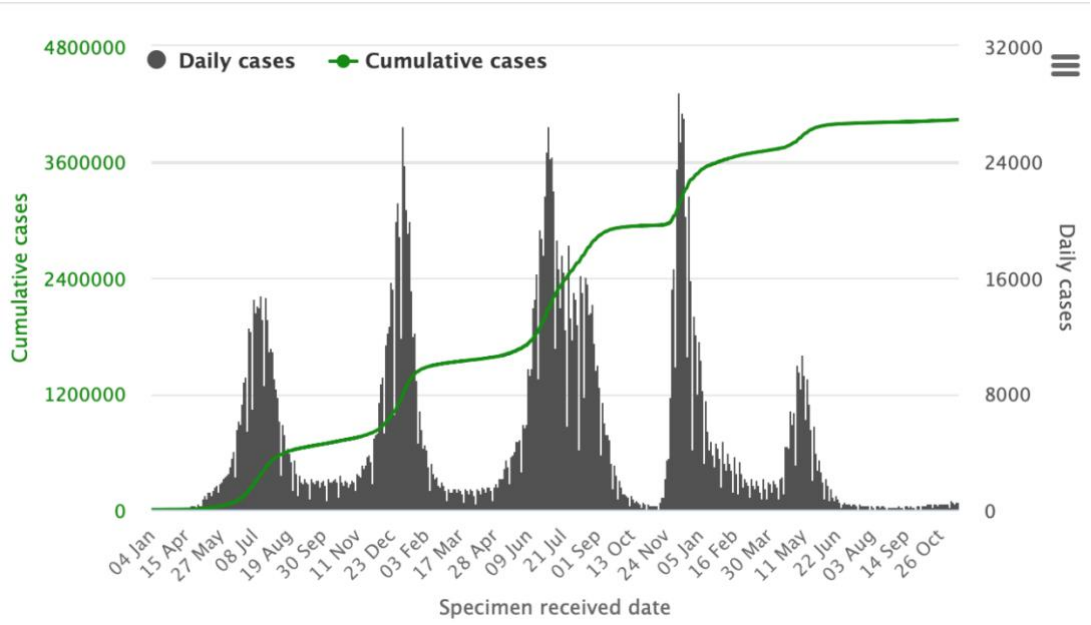

Figure 6: Daily new cases in South Africa showing variant infection waves. Source: https://sacoronavirus.co.za/covid-19-daily-cases/.

1.4.1. Alpha (B.1.1.7)

Alpha B.1.1.7 was first detected in the United Kingdom in December 2020. This variant presented 23 mutations with 17 amino acid changes. By February 22, 2020, B.1.1.7 had been reported in 93 countries and showed a slight decrease in neutralisation escape from natural immunity (1.5 fold) (Abdool Karim and de Oliveira, 2021), defined by the N501Y mutation in the RBD and deletions in the N-terminal domain. This variant emerged early in the epidemic and spread rapidly across the globe (Tegally et al., 2021b) resulting in South Africa's first wave (together with B.1.17).

1.4.2. Beta (B.1.351)

B.1.351 (Beta), initially designated 501Y.V2, was reported in South Africa on December 18, 2020 (Aleem et al., 2022, Cele et al., 2021a) and became the dominant variant, driving more than 95% of infections (Tegally et al., 2021a). By February 22, 2021, B.1.351 was reported in 45 countries (Abdool Karim and de Oliveira, 2021). A threshold of 100,000 cases of COVID-19 was reached approximately 50% quicker in the second wave of infection relative to the first wave (54 vs. 107 days) (Abdool Karim and de Oliveira, 2021). The variant is defined by eight mutations in the spike protein, including three substitutions (K417N, E484K, and N501Y) at residues in the RBD (Tegally et al., 2021a, Greaney et al., 2021) and showed considerable escape from neutralizing antibody immunity of COVID-19 convalescent patients infected with ancestral SARS-CoV-2 (Wibmer et al., 2021, Cele et al., 2021a).

1.4.3. Delta (B.1.617.2)

The B.1.617.2 (Delta) variant was first identified in the state of Maharashtra in late 2020, outcompeting pre-existing lineages (Ferreira et al., 2021). The Delta variant spread rapidly and displaced all other variants in most regions of the world. In South Africa, the Beta variant was displaced around June 2021 (Dhar et al., 2021). Within three months the variant dominated South Africa, causing a resurgence in community transmission, leading to a third wave which was associated with a higher number of deaths (Tegally et al., 2021a). The enhanced transmissibility of this variant has been associated with the L452R, P681R, and T478K substitutions in the RBD (Dhawan et al., 2022). The L452R is shown to have resistance to Bamlanivimab (Liu et al., 2021) and a reduced susceptibility to convalescent/vaccinee plasma (Ferreira et al., 2021). The Delta VoC was associated with a higher viral load, and longer duration of infectiousness because of its ability to escape from natural immunity (Karim and Karim, 2021).

1.4.4. Omicron (B.1.1.529)

In October 2021, while Delta was continuing to exhibit elevated levels of transmission in the Northern Hemisphere, the wave was subsiding in southern Africa. By November 2021, 23 months after the first reported case of COVID-19, genomic surveillance teams in South Africa and Botswana had detected a new SARS-CoV-2 variant, causing a resurgence of infections (Karim and Karim, 2021). Within days of the genome being uploaded, the WHO labelled this a variant of concern, designated Omicron B.1.1.529 . Within 3 weeks the variant was present in more than 87 countries (Viana et al., 2021). B.1.1.529 is shown to be associated with enhanced transmissibility, carrying over 30 mutations in the spike glycoprotein including 69–70del, S371L, K417N, N440K, E484A, Q493R, G496S, N501Y, N856K, N969K mutations (Aleem et al., 2022, Viana et al., 2021). The 69-70del is shown to prevent amplification of one of the three genomes in diagnostic PCR assays, resulting in the S-gene target failure (SGTF) (Meng et al., 2021). The K417N mutation is an ACE-2 binding site mutation, present in Beta, Gamma and Omicron.

Omicron emerged at a time when vaccine immunity was increasing around in the world (Karim and Karim, 2021). The median length of hospitalization for those with breakthrough infections was three days during the Omicron, compared to five to six 6 days in the Beta and Delta waves (Sigal, 2022). Our results were supportive of a scenario in which hybrid immunity formed by Omicron infection combined with vaccination protects against reinfection with variants such as Delta. By contrast, unvaccinated participants infected with Omicron BA.1 only, had low neutralization capacity against the Omicron

BA.2, Beta, Delta, and ancestral viruses (Khan et al., 2022a). Soon after B.1.1.529, sub-lineages BA.4 and BA.5 were once again detected in South Africa with changes relative to Omicron BA.1, including substitutions in the spike RBD. We and others showed escape of BA.4 and BA.5 from immunity elicited from BA.1 infection (Cao et al., 2023, Khan et al., 2022b).

1.4.5. Omicron sub-lineages

In October 2021 two new variant designations were assigned to Omicron; BQ.1, and XBB. BQ.1 is the sub-lineage of BA.5 and XBB is a recombinant of BA.2.10.1 and BA.2.75 subvariants (https://www.who.int/news/item/27-10-2022-tag-ve-statement-on-omicron-sublineages-bq.1-and-xbb). Recombination is indicated by a variant name beginning with an "X" (Rahimi and Talebi Bezmin Abadi, 2022). A noticeable difference between BQ.1 and XBB is the number and location of mutations in the RBD. The XBB variant also has mutations in the N-terminal domain (NTD), indicating it may be able to evade antibodies elicited with the previous infection from BA.2 or BA.5 (Cao et al., 2023). Other Omicron variants that have emerged include BA.4.6, BF.7, BA.2.75.2, and BQ.1.1, all of which have the R346T escape mutation (Miller et al., 2023). Miller et al. (2023) showed that BQ.1.1 escape neutralizing antibodies induced by infection and vaccination more effectively than BA.5. In addition, BQ.1.1 neutralizing antibodies (nAb) titers were 7-fold lower than BA.5 nAb titers in individuals who received the monovalent or bivalent mRNA vaccine boosters.

1.5. Immune Response to SARS-CoV-2

The immune system clears or controls infection and is broadly divided into the innate and adaptive arms (Figure 7). The innate immune system is the first line of defence against spread of the pathogen and acts by slowing down reproduction and spread. The adaptive immune system divides into humoral and cellular immunity and is important for the clearance of infection (Sette and Crotty, 2021). Although both innate and adaptive immunity are linked, they each have different cell types with distinct functions. In the case of SARS-CoV-2, humoral immunity includes antibodies produced by B cells that bind to SARS-CoV-2 spike protein, either neutralizing or eliminating it through other effector mechanisms. Cellular immunity includes virus-specific T cells, which provide long-term immunologic memory and rapidly expand on re-exposure to the antigen. CD8+ T cells directly eliminate virally infected cells (Tan et al., 2022), and CD4+ T cells provide help to support the B cell mediated antibody immune response (Barouch, 2022).

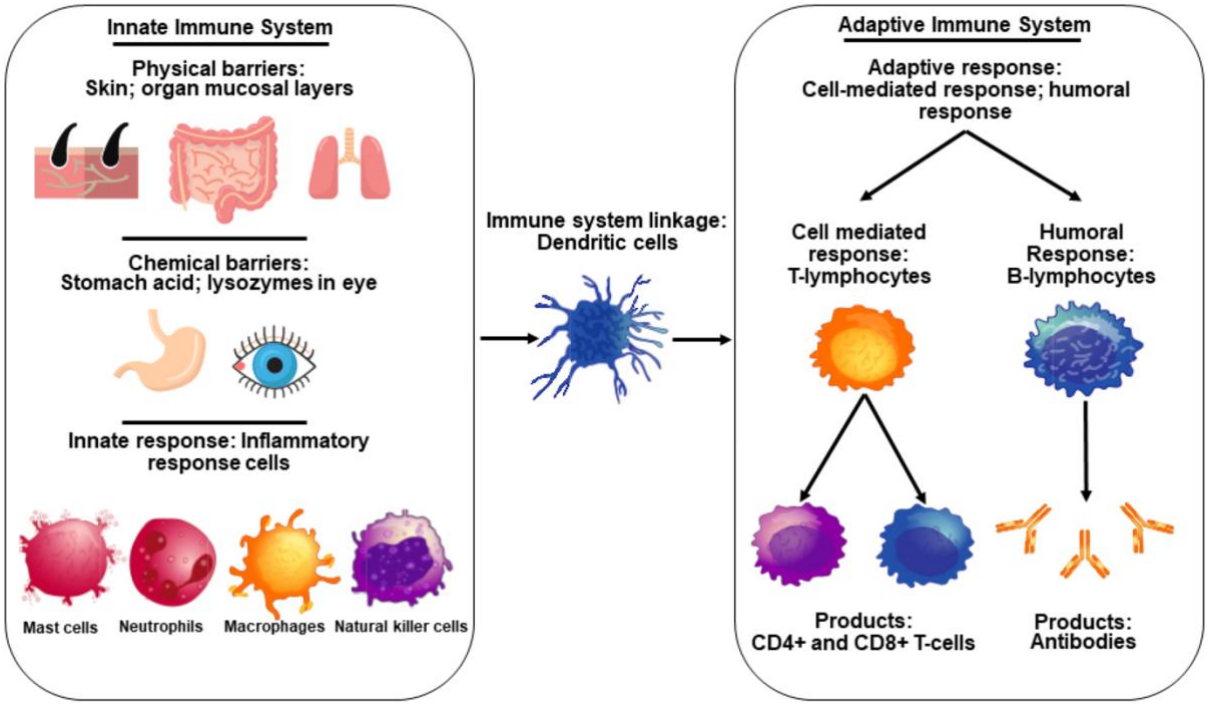

Figure 7: Overview of the immune system (Manna et al., 2022).

1.5.1. Antibody responses to SARS-CoV-2

Neutralizing antibodies can help stop viral infection by different mechanisms that include (a) neutralizing the virus by recognizing epitopes on its surface and (b) improving the activity of other immune components such as complement, phagocytes, and natural killer cells (Lagunas-Rangel and Chávez-Valencia, 2021). Two common antibody assays are used to characterize antibody responses: (a) binding assays, which measure the ability of antibodies in sera to prevent binding of the virus, and (b) neutralization assays, used to measure functional antibody response to measure biological activity throughout the viral replication process (Amanat et al., 2020). Individuals infected with SARS-CoV-2 typically mount both a binding antibody and neutralizing antibody response to antibodies (Huang et al., 2020a, Amanat et al., 2020).

Most individuals infected with SARS-CoV-2 develop neutralizing antibodies that target the viral spike protein (Crawford et al., 2021). Neutralizing antibodies in infected individuals develop rapidly, about 10 days post symptom onset (Long et al., 2020, Sette and Crotty, 2021). Patients generally show a gradual increase in virus-specific IgG and IgM levels until the third week after the onset of symptoms. IgM levels then begin to decrease, while IgG levels continue increasing, keeping IgG S titers stable for approximately three months (Lagunas-Rangel and Chávez-Valencia, 2021). In a well-characterized prospective longitudinal cohort of individuals across a range of disease severity, Crawford et al. (2021),

showed that neutralizing antibody titers waned over 4 months. The decline in nAbs was accompanied by a decline in total antibodies capable of binding the viral spike protein or its RBD. While most focus on antibody levels in blood, Isho et al. (2020), looked at IgG, IgA, and IgM responses to the SARS-CoV-2 spike protein and RBD in serum versus saliva of acute and convalescent patients. The IgG response persisted up to three months after the onset of symptoms and correlated well with serum titers (Isho et al., 2020, Lagunas-Rangel and Chávez-Valencia, 2021).

1.6. SARS-CoV-2 vaccines

Until SARS-CoV-2, vaccine development for coronaviruses was given a low priority (Krammer, 2020). Rising COVID-19 mortality and infection rates expedited vaccine development across the globe (Thakur et al., 2022). The rapid development of multiple COVID-19 vaccines has been a triumph in biomedical research, and billions of vaccine doses have been administered worldwide (Barouch, 2022). To date more than 12.96 billion doses of vaccines (https://ourworldindata.org/covid-vaccinations) have been administered globally with an average of 2.57 million administered daily, indicating that 68.4% of the world population has received at least one dose of a COVID-19 vaccine (Figure 8) .

Vaccine development was initiated when the genetic sequence of the virus became available in early January 2020 and moved at an unprecedented speed (Krammer, 2020). Vaccines based on neutralizing antibodies have a significant drawback in that their viral targets (through mutations) are moving ones (Welsh, 2021). Understanding adaptive immunity to SARS-CoV-2 is therefore important for vaccine development (Grifoni et al., 2020). Owing to their genetic similarities, data from the preclinical development of vaccine candidates for SARS-CoV and MERS-CoV saved a considerable amount of time allowing production processes to be simply adapted from those of existing vaccines or vaccine candidates. As a result, the first clinical trial of a vaccine candidate for SARS-CoV-2 began in March 2020 (NCT04283461), designed to determine the safety, reactogenicity, and immunogenicity of mRNA-1273 (Jackson et al., 2020, Kyriakidis et al., 2021)

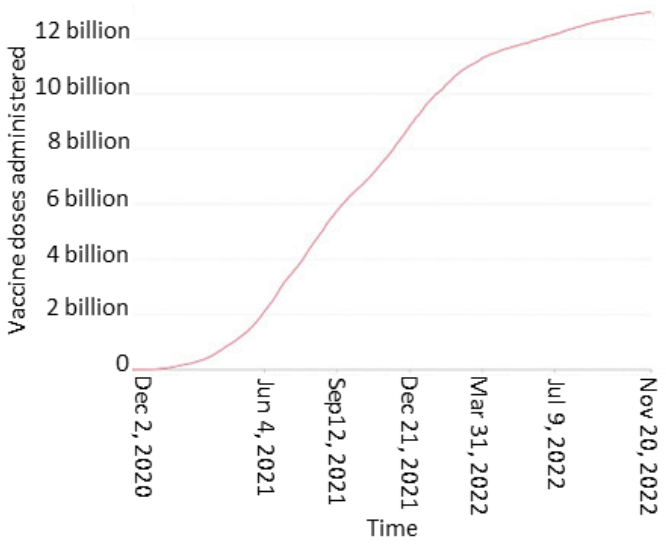

Figure 8: COVID-19 vaccinations administered worldwide. Source: https://ourworldindata.org/covid-vaccinations.

The five common types of platforms utilized to generate COVID-19 vaccines are (a) live-attenuated or inactivated vaccine, (b) protein subunit, (c) viral vector, (d) nucleic acid vaccine (mRNA and plasmid DNA), and (e) virus-like particle vaccine (Thakur et al., 2022). Almost all vaccine candidates except for live attenuated vaccines target the S-gene. Because RNA viruses are known to mutate more frequently than DNA viruses (Masters, 2006), this may impact reactivity with nAbs (Thakur et al., 2022).

1.6.1. mRNA vaccines

The messenger RNA (mRNA) vaccines are relatively new and work by delivering the genetic information for the antigen instead of the antigen, following expression in vaccinated individuals (Welsh, 2021). The two mRNA vaccines that have received emergency use authorization are (a) BNT162b2 (Pfizer/BioNTech) and (b) mRNA-1273 (Moderna) (Thakur et al., 2022, Krammer, 2020). Both encode the spike protein from the Wuhan-Hu-1 variant of SARS-CoV-2. However, emerging variants of SARS-CoV-2 contain spike protein mutations. These could potentially evade nAb responses induced by vaccines (Bordon, 2021). Cele et al. (2021b) and others, (Wilhelm et al., 2022, Garcia-Beltran et al., 2022, Rössler et al., 2022) showed a substantial fold-drop in the neutralization of Omicron compared with the ancestral virus by BNT162b2 vaccine-elicited immunity. Messenger RNA technology has an advantage in that the mRNA sequence of the target in the case of significant mutations can be easily modified and scaled up (Thakur et al., 2022, Krammer, 2020).

1.6.2. Vector vaccines

Non-replicating viral vector vaccines use an engineered adenovirus to deliver the DNA code for the target protein and represent a large group of vaccines in development, most based on the adenovirus vectors (Welsh, 2021, Thakur et al., 2022). All currently approved adenoviral vector vaccines for SARS-CoV-2 target the spike protein (Krammer, 2020). The vaccine is delivered intramuscularly, enters the cells of the vaccinated individual, and expresses the spike protein, triggering an immune response. A major disadvantage of adenoviral vector vaccines is that some people may have pre-existing immunity to adenoviruses, which can decrease the effectiveness of this approach (Welsh, 2021). Exposure to the same adenovirus vaccine again may be cleared by the person's immune system before producing spike protein. This can be circumvented by using different vector types for initial priming and booster vaccines (Zhu et al., 2020a, Mercado et al., 2020, Folegatti et al., 2020, Krammer, 2020, Thakur et al., 2022). Two viral vector vaccines approved for use currently are the chimpanzee adenovirus vectored vaccine; ChAdOx1 nCoV-19 (Folegatti et al., 2020), and a replication-incompetent adenoviral vector based on human adenovirus type 26 vaccine; Ad26.CoV2.S by Janssen (Krammer, 2020). A study by Bekker et al. (2022) and Sadoff et al. (2021) assessed the effectiveness of a single dose of the Ad26.CoV2.S vaccine in healthcare workers in South Africa. The single-dose vaccine showed effectiveness against severe COVID-19 disease and COVID-19-related death after vaccination, against both Beta and Delta variants (Bekker et al., 2022).

1.7. Vaccine roll-out in South Africa

The Ad26.CoV2.S vaccine was rolled out in South Africa on February 17, 2021, through the SISONKE trial (https://www.samrc.ac.za/media-release/south-africa-commences-early-access-vaccine-rollout-healthcare-workers-sisonke). Through this, a single dose of the Ad26.CoV2.S vaccine was made available to healthcare workers before the national roll-out. South Africa also received 39 million doses of the Pfizer vaccine. Most individuals are vaccinated with either one dose of Ad26.CoV2.S (and boosted) or 2 doses of Pfizer/BioNTech BNT162b2.

The total number of vaccinated individuals in South Africa is approximately 20.54 million (Figure 9a), as of November 17, 2022, accounting for 31.6% of the South African population . An individual is deemed vaccinated if they received one shot of the Ad26.CoV2.S vaccine or two doses of the BNT162b2. The total number of individuals vaccinated with Ad26.CoV2.S is 7,870,436 with 1,446,461 receiving a second booster dose, and 12,676,537 with Pfizer/BioNTech BNT162b2. The total number of vaccines administered including booster doses is approximately 38 million (Figure 9b) with fewer than 200 individuals currently vaccinated on a daily basis.

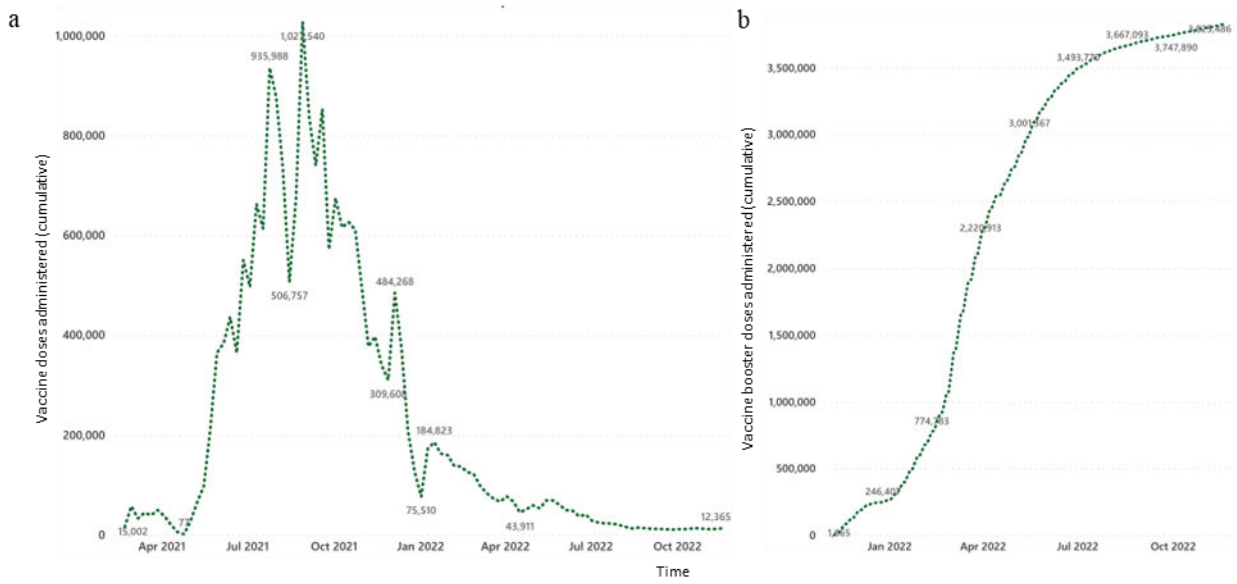

Figure 9: Vaccination in South Africa. Source: https://sacoronavirus.co.za/latest-vaccine-statistics/.

1.7.1. Vaccination among people living with HIV (PLWH)

South Africa contributes to about 18% of the global HIV burden with an estimated 8.2 million South Africans living with HIV, as of 2021 (https://www.unaids.org/en/regionscountries/countries/southafrica). This represents 13.7% of the national population with an HIV prevalence rate of 19.7% across the 15-49 age group (Kharsany et al., 2018, Govere-Hwenje et al., 2022). An important consideration in this geographical setting is the high prevalence of co-infection with SARS-CoV-2 and HIV. Treatment with antiretroviral therapy (ART) effectively suppresses HIV and can lead to better outcomes (Samandari et al., 2017), however, poor adherence can lead to advanced HIV disease and immunosuppression (Khan et al., 2021). A recent study (Cele et al., 2022) mapped the evolution of SARS-CoV-2 over 6 months in a person with advanced HIV and showed that non-adherence to ART can lead to long-term infection of the SARS-CoV-2 virus.

Worldwide, PLWH form a vulnerable population who may be at risk for worse clinical outcomes from COVID-19 and could benefit from vaccination (Govere-Hwenje et al., 2022). COVID-19 vaccines became available to South Africans in February 2021. However, only 31% of the population is currently vaccinated . Vaccine hesitancy was reported as one of the 10 global threats to health in 2019 (https://www.who.int/news-room/spotlight/ten-threats-to-global-health-in-2019) and one of the reasons for poor vaccination rates among South Africans (Govere-Hwenje et al., 2022). In South Africa, a total of approximately 24 000 immunocompromised individuals have been vaccinated (Figure 10)

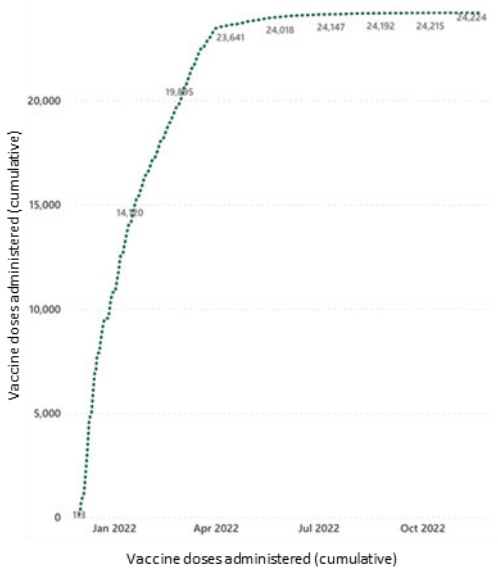

Figure 10: Total number of immunocompromised individuals vaccinated in South Africa. Source: https://sacoronavirus.co.za/latest-vaccine-statistics/.

Concerns stemming from the emergence of new variants are their effects on viral transmissibility, disease severity, reinfection rates due to escape, and vaccine effectiveness or escape from vaccine-induced immunity (Abdool Karim and de Oliveira, 2021). The aim of this thesis was to understand how SARS-CoV-2 evolution, and hybrid immunity impacts viral transmission and COVID-19 disease outcome. This was explored by examining the effect of SARS-CoV-2 variants and co-infection with HIV on the neutralizing antibody response elicited by SARS-CoV-2 infection and vaccination.

CHAPTER 2: IMMUNOGENICITY OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 (SARS-COV-2) INFECTION AND AD26.COV2.S VACCINATION IN PEOPLE LIVING WITH HUMAN IMMUNODEFICIENCY VIRUS (HIV)

In this chapter, the immunogenicity of SARS-CoV-2 infection with and without vaccination in PLWH was compared, in comparison to HIV negative individuals. People living with HIV have been reported to have a higher risk of more severe COVID-19 disease and death (Yang et al., Boulle et al., 2020). The ability of the Ad26.CoV2.S vaccine to elicit neutralizing antibody activity against the Delta variant in PLWH relative to HIV negative individuals was assessed. The effects of HIV status and suppression on Delta neutralization response in SARS-CoV-2–infected unvaccinated participants was also examined. The neutralization response of the Delta variant following Ad26.CoV2.S vaccination in people with well controlled HIV was not inferior to HIV negative participants, irrespective of past SARS-CoV-2 infection. However, unvaccinated individuals infected with SARS-CoV-2, and HIV-viremic showed a reduction in neutralisation capacity of SARS-CoV-2. These results underscore the ability of hybrid immunity to compensate for the lower neutralizing immunity elicited in PLWH by infection alone.

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Immunogenicity of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection and Ad26.CoV2.S Vaccination in People Living With Human Immunodeficiency Virus (HIV)

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Background. People living with HIV (PLWH) have been reported to have a higher risk of more severe COVID-19 disease and death. We assessed the ability of the Ad26.CoV2.S vaccine to elicit neutralizing activity against the Delta variant in PLWH relative to HIV-negative individuals. We also examined effects of HIV status and suppression on Delta neutralization response in SARS-CoV-2–infected unvaccinated participants.

Methods. We enrolled participants who were vaccinated through the SISONKE South African clinical trial of the Ad26.CoV2.S vaccine in healthcare workers (HCWs). PLWH in this group had well-controlled HIV infection. We also enrolled unvaccinated participants previously infected with SARS-CoV-2. Neutralization capacity was assessed by a live virus neutralization assay of the Delta variant.

Results. Most Ad26.CoV2.S vaccinated HCWs were previously infected with SARS-CoV-2. In this group, Delta variant neutralization was 9-fold higher compared with the infected-only group and 26-fold higher relative to the vaccinated-only group. No decrease in Delta variant neutralization was observed in PLWH relative to HIV-negative participants. In contrast, SARS-CoV-2–infected, unvaccinated PLWH showed 7-fold lower neutralization and a higher frequency of nonresponders, with the highest frequency of nonresponders in people with HIV viremia. Vaccinated-only participants showed low neutralization capacity.

Conclusions. The neutralization response of the Delta variant following Ad26.CoV2.S vaccination in PLWH with well-controlled HIV was not inferior to HIV-negative participants, irrespective of past SARS-CoV-2 infection. In SARS-CoV-2–infected and nonvaccinated participants, HIV infection reduced the neutralization response to SARS-CoV-2, with the strongest reduction in HIV viremic individuals.

Keywords. SARS-CoV-2; Ad26.CoV2.S vaccines; immunogenicity; neutralization; HIV viremia.

South Africa has a high burden of human immunodeficiency virus (HIV) infection [1] and recent studies observed coronavirus disease 2019 (COVID-19) disease severity [2, 3] and mortality risk [3, 4] are increased among people living with HIV (PLWH). HIV interferes with protective vaccination against multiple pathogens, usually through the decreased effectiveness of the antibody response [5–9]. HIV infection reduces the number of CD4 T cells [10], the primary HIV target cells in different anatomical compartments [11]. Reduced CD4 T-cell numbers correlate with reduced concentrations of antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [12].

The effects of HIV status on vaccine efficacy are still being determined. While the number of PLWH participants was very

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small, there was no efficacy of the Novavax NVX-CoV2373 vaccine in PLWH [13]. SARS-CoV-2 vaccine efficacy may also be reduced due to having to cross-neutralize a SARS-CoV-2 variant. For example, infection with the Beta variant [14–16] was associated with a dramatic decrease in the ability the AstraZeneca ChAdOx vaccine to elicit an effective neutralization response [17]. The effect of HIV status on the protection mediated by the adenovirus vectored Ad26.CoV2.S vaccine is yet unknown.

SARS-CoV-2 neutralization by antibodies correlates with SARS-CoV-2 vaccine efficacy [18] and may be a predictor of vaccine efficacy where efficacy data are not yet available. Two studies examining neutralization elicited by the ChAdOx1 nCoV-19 chimpanzee adenovirus vectored vaccine in PLWH with well-controlled HIV observed comparable anti–SARS-CoV-2 spike receptor binding domain (RBD) antibody levels [19, 20]. Decreased neutralization of the ancestral spike sequence in PLWH was observed in 1 study, but 95% confidence intervals (CIs) for neutralization overlapped between PLWH and HIV-negative participants [20]. Interestingly, when neutralization of the Beta SARS-CoV-2 variant was examined in vaccinated participants with detectable neutralization of ancestral virus, 50% of PLWH retained some neutralization activity against the Beta variant compared with only 15% of HIV-negative participants [20].

Several studies examined the effect of HIV on Pfizer-BNT162b2 mRNA vaccine–elicited SARS-CoV-2 spike binding antibodies and neutralization. Most studies found no significant effect of HIV status when testing participants with well-controlled HIV infection [21–24]. One study found that there was also no significant difference in BNT162b2-elicited SARS-CoV-2 binding antibody concentrations to the Beta, Alpha, and Gamma variants in PLWH [24]. A second study found that PLWH with CD4 counts of less than 300 cells/μL (HIV viral load [VL] was unreported) mounted similar anti–SARS-CoV-2 binding antibody responses relative to HIV-negative participants and PLWH with CD4 counts greater than 300 cells/μL [22]. In contrast, another study testing the effect of low CD4 counts showed that anti–SARS-CoV-2 spike receptor binding domain antibodies elicited by BNT162b2 were dramatically lower in PLWH with CD4 counts less than 250 cells/μL [25].

The effect of HIV status on vaccine immunogenicity was examined for the Beijing Institute of Biological Products BIBP-CorV inactivated virus vaccine by measuring binding antibodies and neutralization in a surrogate neutralization assay [26]. This vaccine is administered in 2 doses. Despite the overall conclusion that the vaccine is immunogenic in PLWH, some differences were found. First, PLWH had significantly lower spike RBD binding antibodies after the first (but not the second) dose of the vaccine. Second, PLWH with a CD4:CD8 ratio of less than 0.6, likely indicating HIV-mediated CD4 depletion, showed lower binding and neutralizing antibody responses relative to PLWH with CD4:CD8 greater than 0.6. Whether the participants with CD4:CD8 less than 0.6 were also viremic was not reported. However, about one-third of participants in the study had a detectable HIV VL (defined as >20 HIV RNA copies/mL).

While vaccine elicited neutralization in PLWH vaccinated with the single-dose Johnson and Johnson Ad26.CoV2.S has not been previously reported, data from HIV-negative participants in the SISONKE trial of the Ad26.CoV2.S vaccine in healthcare workers (HCWs) [27] showed moderate neutralization in vaccinated participants, which was enhanced when vaccination was on the background of previous SARS-CoV-2 infection [28].

Here we investigated whether the Ad26.CoV2.S vaccine elicits a comparable neutralizing response to the Delta variant [14] in PLWH relative to HIV-negative study participants using a live virus neutralization assay. We compared the results to SARS-CoV-2–infected unvaccinated participants. The Delta variant was the dominant variant in South Africa and globally at the time when the neutralization assays were performed [14, 29]. We observed that well-controlled HIV infection did not reduce the Ad26.CoV2.S vaccine–elicited neutralization response. In SARS-CoV-2–infected unvaccinated participants, we observed that HIV infection did interfere with the neutralization response to SARS-CoV-2 and interference was strongest in HIV viremic PLWH.

METHODS

Ethical Statement

Blood samples were obtained after informed consent from Ad26.CoV2.S vaccinees and adults with polymerase chain reaction (PCR)–confirmed SARS-CoV-2 infection enrolled in a prospective cohort study approved by the Biomedical Research Ethics Committee at the University of KwaZulu–Natal (reference BREC/00001275/2020).

Cells and Virus Expansion

Vero E6 cells (ATCC CRL-1586, obtained from Cellonex in South Africa) were propagated in complete Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum (Hylone) with 1% each of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sodium pyruvate, L-glutamine, and nonessential amino acids (Sigma-Aldrich). All work with live virus was performed in Biosafety Level 3 containment using protocols approved by the Africa Health Research Institute Biosafety Committee. We used angiotensin-converting enzyme 2 (ACE2)–expressing H1299-E3 cells for the initial isolation (P1 stock) followed by passaging in Vero E6 cells (P2 and P3 stocks, where P3 stock was used in experiments). Viral supernatant was aliquoted and stored at −80°C. The Delta variant virus was isolated as previously described

[14]. Detailed information is found in the Supplementary Methods.

Microneutralization Using the Focus-Forming Assay

Vero E6 cells were plated in a 96-well plate (Corning) at 30,000 cells per well 1 day pre-infection. Plasma was separated from ethylenediaminetetraacetic acid (EDTA)–anticoagulated blood by centrifugation at 500 x *g* for 10 min and stored at −80°C. Aliquots of plasma samples were heat-inactivated at 56°C for 30 minutes and clarified by centrifugation at 10,000 rcf for 5 minutes. GenScript A02051 anti-spike neutralizing monoclonal antibody was added as a positive control to 1 column of wells. Final plasma dilutions were 1:25, 1:50, 1:100, 1:200, 1:400, 1:800, and 1:1600. Virus stocks were used at approximately 50–100 focus-forming units per microwell and added to diluted plasma. Antibody–virus mixtures were incubated for 1 hour at 37°C, 5% CO_2. Cells were infected with 100 µL of the virus–antibody mixtures for 1 hour, then 100 µL of overlay (1× Roswell Park Memorial Institute [RPMI] 1640 [Sigma-Aldrich, R6504] with 1.5% carboxymethylcellulose [Sigma-Aldrich, C4888]) was added without removing the inoculum. Cells were fixed 18 hours post-infection using 4% paraformaldehyde (Sigma-Aldrich) for 20 minutes. Foci is stained with a rabbit anti-spike monoclonal antibody (BS-R2B12; GenScript A02058) at 0.5 µg/mL in a permeabilization buffer containing 0.1% saponin (Sigma-Aldrich), 0.1% bovine serum albumin (BSA; Sigma-Aldrich), and 0.05% Tween-20 (Sigma-Aldrich) in phosphate-buffered saline (PBS). Plates were incubated with primary antibody overnight at 4°C, then washed with wash buffer containing 0.05% Tween-20 in PBS. Secondary goat anti-rabbit horseradish peroxidase (Abcam ab205718) antibody was added at 1 µg/mL and incubated for 2 hours at room temperature with shaking. TrueBlue peroxidase substrate (SeraCare 5510-0030) was then added at 50 µL per well and incubated for 20 minutes at room temperature. Plates were imaged using the ImmunoSpot Ultra-V S6-02-6140 Analyzer Elispot instrument with BioSpot Professional built -in image analysis (Cellular Technology Ltd).

Multi-Epitope Protein Microarray

ImmuSAFE COVID-19 Array slides (Sengenics Corporation, Singapore) were used to measure the anti–SARS-CoV-2 immunoglobulin G (IgG) antibodies against SARS-CoV-2 nucleocapsid. The microarray-based assays were performed as previously described [30] with modifications as described in the Supplementary Methods. As a threshold, the mean plus 2 standard deviations from pre-pandemic control signal was used.

Statistics and Fitting

All statistics and fitting were performed using MATLAB v.2019b. Neutralization data were fit to $Tx = 1/1 + (D/ID_{50})$. Here, Tx is the number of foci normalized to the number of foci in the absence of plasma on the same plate at dilution D and ID_{50} is the plasma dilution giving 50% neutralization: focus reduction neutralization titer ($FRNT_{50}$) = $1/ID_{50}$. Values of $FRNT_{50}$ <1 are set to 1 (undiluted), the lowest measurable value. We note that the most concentrated plasma dilution was 1:25 and therefore $FRNT_{50}$ <25 were extrapolated.

RESULTS

We tested SARS-CoV-2 neutralization in Ad26.CoV2.S-vaccinated HIV-negative and PLWH participants enrolled in the SISONKE trial, whose aim was to monitor the effectiveness of the single-dose Ad26.COV2.S vaccine among 500 000 HCWs in South Africa (ClinicalTrials.gov number NCT04838795). The SISONKE trial administered only the Ad26.CoV2.S vaccine and started in February 2021. It was the first widespread vaccination effort in South Africa. No other group in addition to HCWs was enrolled. Out of 99 Ad26.COV2.S-vaccinated participants enrolled in our study, 73 (73%) were HIV-negative and 26 (26%) were PLWH. As expected, HCWs are well linked to care and all but 1 vaccinated PLWH showed an undetectable HIV VL (Table 1). As a comparison group, we also enrolled unvaccinated participants with prior documented SARS-CoV-2 infection. This group (n = 62) had 28 (45%) HIV-negative participants and 34 (55%) PLWH. In the unvaccinated PLWH group, 29% had a detectable HIV VL, with a median of 3060 (1224–30 160) HIV RNA copies/mL (Table 1). We also used pre-pandemic stored plasma samples as controls (Supplementary Table 1).

We categorized participants into vaccinated only, previously infected and vaccinated, and SARS-CoV-2–infected unvaccinated. The time post-infection of samples from the infection-only group was matched as closely as possible to the median time post-infection in the infected/vaccinated group (range, 6–10 months) (Table 1). Vaccination occurred approximately 2 months before blood samples were taken to test neutralization in vaccinated participants (Table 1). We used a record of a SARS-CoV-2–positive quantitative PCR (qPCR) as an indication of previous SARS-CoV-2 infection for all SARS-CoV-2–infected unvaccinated participants and vaccinated participants where such a record was available. To account for asymptomatic or unreported SARS-CoV-2 infection in vaccinated participants, we tested for the presence from SARS-CoV-2 nucleocapsid protein antibodies [30], which are made against the nucleocapsid protein produced in infection but not delivered by Ad26.CoV2.S vaccination. Therefore, a participant was considered previously infected if either nucleocapsid antibodies were detected (Supplementary Figure 1) or a previous positive qPCR for SARS-CoV-2 existed. Of the vaccinated participants, 68% were found to be previously infected with SARS-CoV-2 (Supplementary Figure 1).

The Delta variant became dominant in the province of KwaZulu-Natal, the location of this study, in July 2021 (14). We

Table 1. Study Participant Characteristics

	Infected Unvaccinated			Infected and Vaccinated			Vaccinated Only		
	All	HIV−	HIV+	All	HIV−	HIV+	All	HIV−	HIV+
Number of participants	62	28 (45.2%)	34 (54.8%)	67	49 (73.1%)	18 (26.9%)	32	24 (75.0%)	8 (25.0%)
Age, y	44 (39–57)	57 (46–64)	41 (35–45)	46 (40–52)	46 (40–52)	47 (42–51)	45 (39–52)	48 (42–55)	39 (36–42)
Days post-infection	188 (120–278)	192 (108–279)	187 (122–277)	235 (141–306)	230 (134–303)	304 (187–333)	…	…	…
Days post-vaccination	…	…	…	48 (34–81)	48 (34–80)	51 (34–86)	74 (50–84)	74 (44–85)	74 (61–82)
Male sex	12 (19.4%)	5 (17.9%)	7 (20.6%)	2 (3.0%)	2 (4.1%)	0 (0.0%)	1 (3.1%)	0 (0.0%)	1 (12.5%)
Number HIV viremic	…	…	10 (29.4%)	…	…	1 (5.6%)	…	…	0 (0.0%)
HIV viral load	…	…	3060 (1224–30 160)	…	…	3219	…	…	…
Years of ART	…	…	11 (5–15)	…	…	7 (5–12)	…	…	5 (4–11)
CD4 count cells/μL	792 (513–1027)	991 (807–1179)	581 (328–794)	967 (784–1325)	1033 (877–1424)	852 (730–1184)	1199 (853–1368)	1215 (1101–1413)	735 (458–863)
CD4:CD8 ratio	1.1 (0.7–1.2)	1.6 (1.3–2.1)	0.8 (0.4–1.1)	1.6 (1.1–2.2)	1.7 (1.4–2.3)	1.1 (0.8–1.2)	1.8 (1.2–2.1)	1.9 (1.2–2.3)	1.1 (0.4–1.2)

All values are medians (IQR) or n (%). Number HIV viremic is the number of PLWH with HIV RNA >40 copies/mL of total PLWH. Median HIV viral load is for HIV viremic participants only. Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range; PLWH, people living with HIV.

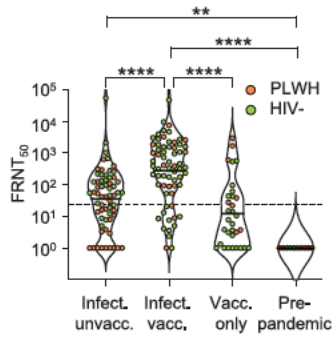

Figure 1. Effect of previous SARS-CoV-2 exposure on neutralization capacity elicited by Ad26.CoV2.S. Violin plots of neutralization capacity of Delta variant as $FRNT_{50}$ in SARS-CoV-2–infected unvaccinated, infected and vaccinated, vaccinated-only, and pre-pandemic participants. PLWH are represented by orange points and HIV-negative participants by green points. Horizontal lines represent GMT. Participant numbers per category were n = 62 (34 PLWH, 28 HIV) for infected unvaccinated, n = 67 (18 PLWH, 49 HIV−) for infected vaccinated, and n = 32 (8 PLWH, 24 HIV−) for vaccinated only. *P* values are as follows: ** <.01, **** <.0001 as determined by the Kruskal-Wallis test with Dunn multiple hypothesis correction. The dashed horizontal line denotes most concentrated plasma tested. Abbreviations: $FRNT_{50}$, focus reduction neutralization test (50 is the plasma dilution giving 50% neutralization); GMT, geometric mean titer; HIV, human immunodeficiency virus; Infect., infected; PLWH, people living with HIV; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; unvacc., unvaccinated; Vacc., vaccinated.

used a live virus neutralization assay of the Delta variant since it is currently the most widespread variant in South Africa and globally. We note that none of the participants with a record of previous infection were infected in the Delta infection wave (Supplementary Table 2).

We observed that SARS-CoV-2–infected-only participants had low but detectable SARS-CoV-2 Delta variant neutralization measured in a focus reduction neutralization test (FRNT), where $FRNT_{50}$ is the inverse of the dilution required for 50% neutralization (Figure 1). Neutralization was significantly higher in the group receiving Ad26.CoV2.S vaccination relative to the infected-only group (geometric mean titer [GMT] of 307 [95% CI, 167–562] vs 36 [95% CI, 20.8–63.8], a 9-fold increase; $P < .0001$). Neutralization in the vaccinated/infected group was also 26-fold higher than in the vaccinated-only group (GMT = 12 [5.1–28.7], $P < .0001$), although the $FRNT_{50}$ in the latter was below the lowest dilution tested and therefore extrapolated. While neutralization in the infected-only group was higher than in the vaccinated-only group, the difference was not significant.

In the infected-only group, neutralization of the Delta variant was 7-fold lower in PLWH relative to HIV-negative participants (Figure 2A) (GMT = 105 [50.4–218] for HIV-negative, 15 [7.3–31.6] for PLWH; $P = .001$). In contrast, there was no significant difference in vaccine-elicited neutralization in PLWH versus HIV-negative participants who received the vaccine following SARS-CoV-2 infection (Figure 2B). In vaccinated-only participants, PLWH seemed to have a stronger vaccine-elicited neutralization of Delta with borderline significance (Figure 2C)

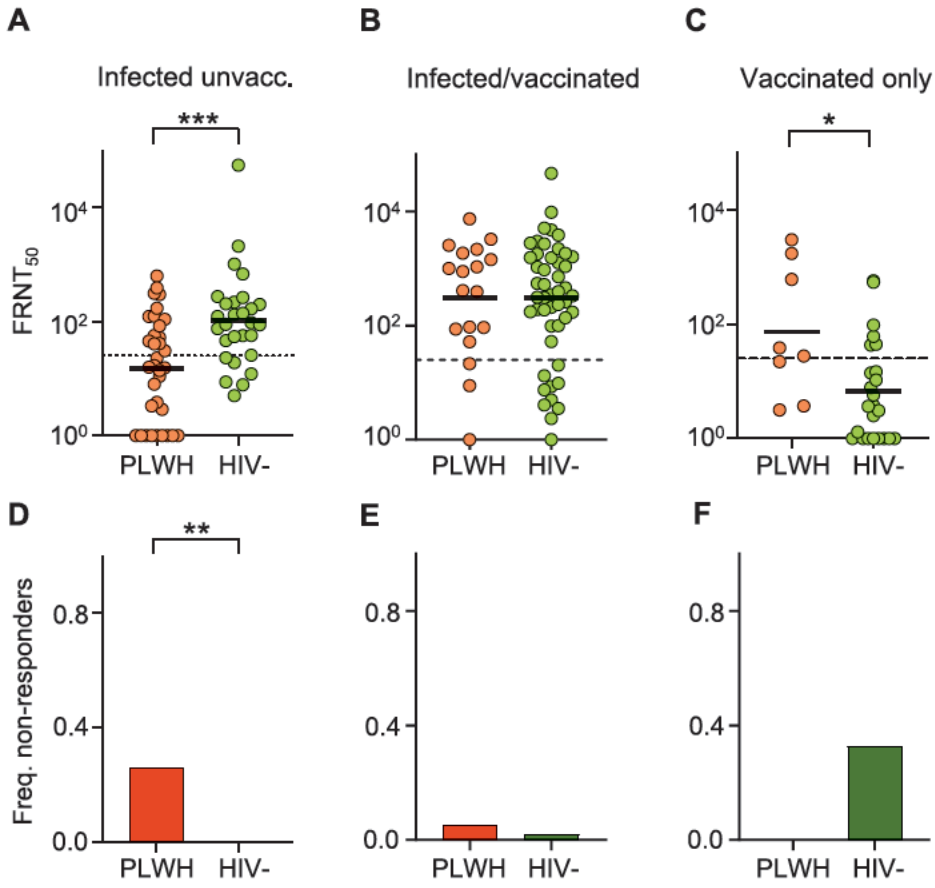

Figure 2. Effect of HIV status on neutralization capacity elicited by Ad26.CoV2.S. (*A–C*) Neutralization capacity as $FRNT_{50}$ for Delta variant neutralization in SARS-CoV-2–infected unvaccinated (*A*), infected and vaccinated (*B*), and vaccinated only (*C*) participants. Solid horizontal lines represent GMT and dashed horizontal lines represent most concentrated plasma used. (*D–F*) frequency of participants with no detectable Delta variant neutralization (nonresponders) in SARS-CoV-2–infected unvaccinated (*D*), infected and vaccinated (*E*), and vaccinated-only (*F*) participants. *P* values are as follows: * <.05, ** <.01, *** <.001, as determined for panels *A–C* by the Mann–Whitney *U* test and for panels *D–F* by Fisher's exact test. Abbreviations: Freq., frequency; $FRNT_{50}$, focus reduction neutralization test (50 is the plasma dilution giving 50% neutralization); GMT, geometric mean titer; HIV, human immunodeficiency virus; PLWH, people living with HIV; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; unvacc., unvaccinated.

(GMT = 6 [2.8–15.4] for HIV-negative, 73 [7.9–677] for PLWH; *P* = .02).

We next examined each group for nonresponders, defined as no detectable neutralization of Delta variant neutralization in the live virus neutralization assay ($FRNT_{50}$ = 1 in Figure 2A-C). The infected-only PLWH showed a frequency of 26.5% of nonresponders while there were no nonresponders in HIV-negative participants, a significant difference (*P* = .0029) (Figure 2D). In contrast, the frequency of nonresponders was only 5.6% in PLWH and 2.0% in HIV-negative individuals in the vaccinated/SARS-CoV-2–infected group. The difference between PLWH and HIV-negative participants in the vaccinated, previously infected group was not significant (*P* = .47) (Figure 2E). In the vaccinated-only group, there were 33.3% nonresponders in the HIV-negative group and none in PLWH, but the difference was nonsignificant (*P* = .082) (Figure 2F).

We next determined the effect of HIV suppression in the SARS-CoV-2–infected-only group (the number of HIV viremic participants in the vaccinated groups was too small for analysis). In this group, 29.4% of PLWH participants had detectable HIV viremia (Table 1), compared with 5.6% in the infected/vaccinated group and none in the vaccinated-only group. There was a lower $FRNT_{50}$ in the infected-only viremic versus HIV-suppressed PLWH (GMT, 6 in HIV viremic vs 22 in suppressed) but this was nonsignificant (Figure 3A) (*P* = .13). The frequency of nonresponders in the HIV viremic subset was 60.0%, while it was 13.0% in HIV-suppressed PLWH, which was significant (Figure 3B) (*P* = .0088; odds ratio, 10.5; 95% CI, 1.8–47.0). However, despite HIV suppression by antiretroviral therapy (ART), there was lower neutralization of the Delta variant in SARS-CoV-2–infected-only, HIV-suppressed PLWH relative to HIV-negative participants (Supplementary Figure 2*A*), although the difference in the fraction of nonresponders became nonsignificant (Supplementary Figure 2*B*).

CD4 T-cell count may be an important determinant of the immune response. The CD4 count was lower in the infected-only

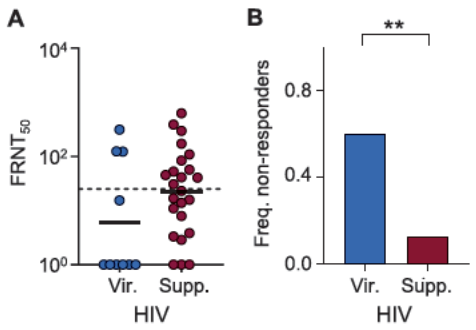

Figure 3. Effect of HIV viremia on neutralization capacity in infected unvaccinated participants. (*A*) Neutralization capacity as $FRNT_{50}$ for Delta variant neutralization in SARS-CoV-2–infected unvaccinated HIV viremic (n = 10) versus infected unvaccinated HIV-suppressed (n = 24) participants. The dashed horizontal line represents most concentrated plasma used. (*B*) Frequency of nonresponders in panel *A*. *P* values are as follows: *P* = .13 for (*A*) by the Mann–Whitney *U* test and *P* = .0088 for (*B*) by Fisher's exact test. Abbreviations: Freq., frequency; $FRNT_{50}$, focus reduction neutralization test (50 is the plasma dilution giving 50% neutralization); HIV, human immunodeficiency virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Supp., suppressed; Vir., viremic.

group (reflecting a higher fraction of PLWH) and was lower in PLWH relative to HIV-negative participants in all groups (Supplementary Figure 3). In the infected-only group, there was a significant correlation between higher CD4 count and higher neutralization (r = 0.36, P = .0045) (Figure 4A). This correlation was closely associated with HIV status, with the lower CD4 counts being in PLWH. There were no significant correlations between CD4 T-cell count and neutralization in the infected vaccinated or vaccinated-only groups (Figure 4B and 4C).

DISCUSSION

Our results are consistent with a noncompromised neutralization response to Ad26.CoV2.S vaccination in PLWH. We note that the vaccinated HCW PLWH tested in our study showed well-controlled HIV infection and relatively high CD4 counts. Ad26.CoV2.S uses the ancestral spike sequence. Moreover, all participants with documented previous infection were infected before the emergence of Delta. Therefore, the neutralization capacity we tested was cross-neutralization of Delta by an antibody response elicited to either ancestral spike (vaccine) or ancestral or Beta variant strains (previous infection).

SARS-CoV-2 antibody levels decay post-infection and vaccination after about the first month, with a half-life of approximately 2 months [18]. The interval between infection and sampling was shorter in the infected-only (median, 6.3 months) versus the vaccinated, previously infected (7.8 months) group. It would therefore be expected that infection-elicited neutralization would be higher in the infected-only group if vaccination had no effect. Instead, vaccinated and previously infected participants had 9-fold higher Delta variant neutralization compared with the infected-only group, indicating that vaccination boosted the neutralization response and more than compensated for the longer time post-infection. In the comparison between the vaccinated and vaccinated previously infected group, the vaccinated-only group was sampled later post-vaccination (median, 2.5 vs 1.6 months for vaccinated and infected). However, given a 2-month half-life, the difference in timing does not account for the 26-fold decrease in neutralization in the vaccinated-only group. It is better explained by vaccine boosting of neutralizing immunity acquired through SARS-CoV-2 infection.

The higher neutralization in vaccinated-only PLWH relative to HIV-negative participants was surprising. However, the number of participants in the comparison was small, there was a wide dispersion in $FRNT_{50}$ values, and the vaccinated-only

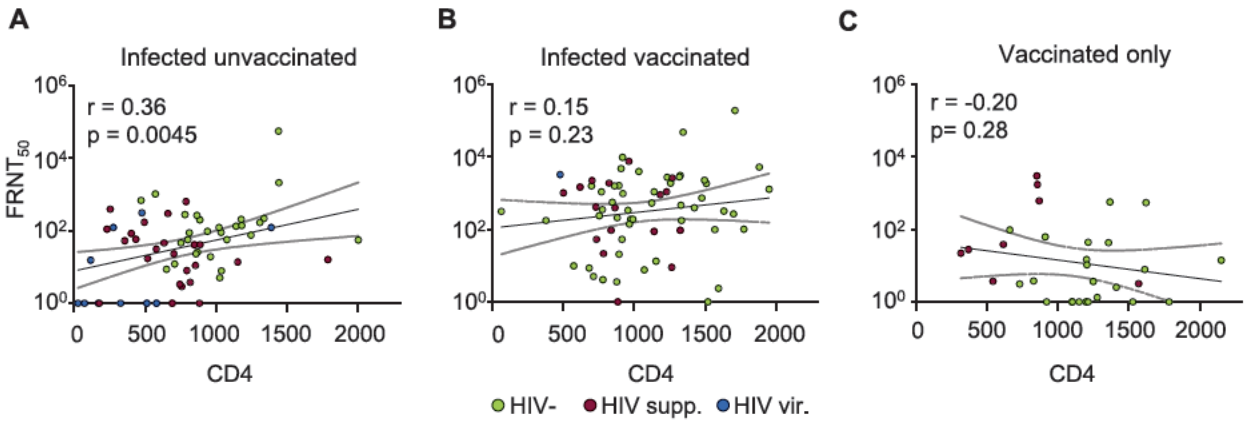

Figure 4. Correlation between CD4 count and neutralization capacity. Pearson correlation of PRNT50 versus CD4 count in infected unvaccinated (*A*), infected and vaccinated (*B*), and vaccinated-only (*C*) participants. Solid lines represent linear regression and upper and lower lines represent 95% confidence intervals. *r* is the Pearson correlation coefficient. Green points are HIV-negative participants, purple points are PLWH with suppressed HIV viremia, and blue points are HIV viremic PLWH. Abbreviations: $FRNT_{50}$, focus reduction neutralization test; HIV, human immunodeficiency virus; PLWH, people living with HIV; supp., suppressed; vir., viremic.

PLWH were younger, perhaps accounting for the better response [31]. Therefore, caution should be used in interpreting these data. A ChAdOx vaccine study previously reported a higher fraction of PLWH participants with well-controlled HIV who detectably cross-neutralized the Beta variant relative to HIV-negative participants, but this, too, was based on low participant numbers [20]. Consistent with results in HIV-negative participants [28], previous SARS-CoV-2 infection enhanced the Ad26.CoV2.S neutralization response.

The effect of HIV status in both the vaccinated-only and vaccinated infected groups contrasts with the infected unvaccinated group, which showed a deleterious effect of HIV infection on neutralization of the Delta variant and an increased number of nonresponders, especially among PLWH with detectable HIV viremia, where the fraction of nonresponders was approximately 5-fold higher than in HIV-suppressed PLWH. However, even in HIV-suppressed PLWH, the neutralization response to Delta was lower. SARS-CoV-2-infected, unvaccinated participants were also the only group where a moderate but significant correlation between CD4 T-cell count and Delta neutralization was detected. We could not examine the effects of HIV viremia on the Ad26.CoV2.S neutralization response in our current study because the SISONKE trial, the first large-scale vaccination effort in South Africa, vaccinated only HCWs, who have good linkage to care and therefore well-suppressed HIV. Future studies will determine the effect of HIV viremia and compare Ad26.CoV2.S with BNT162b2 as the broader population is being vaccinated in South Africa with Ad26.CoV2.S or BNT162b2.

Limitations of this study are that we did not examine the T-cell response or the effect of HIV viremia and low CD4 count on vaccine-mediated neutralization. Also, the number of vaccinated participants without previous SARS-CoV-2 infection, especially in the PLWH group, was small. Both antibody and T-cell responses are critical for effective control and clearance of SARS-CoV-2. Milder COVID-19 disease outcome correlates with a robust T-cell response [32, 33]. If HIV infection dysregulates the T-cell response, it may cause the reported increased COVID-19 disease severity in PLWH [2].

Overall, the results indicate that vaccination with Ad26.CoV2.S has a benefit in terms of conferring SARS-CoV-2 neutralization capacity in PLWH from South Africa with well-suppressed HIV infection.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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CHAPTER 3: OMICRON INFECTION ENHANCES DELTA ANTIBODY IMMUNITY IN VACCINATED PERSONS

Soon after the completion of the previous experiments, Omicron BA.1 emerged in South Africa (Viana et al., 2021). The virus was isolated by Cele et al. (2021b). The extent to which Omicron immunity with and without vaccination, protects against the previously dominant Delta (B.1.617.2) variant as well as other variants was unknown. The neutralization capacity of 39 participants infected with Omicron BA.1 was measured against previous SARS-CoV-2 variants. Individuals were categorized into two groups, with a hybrid immunity group vaccinated with Ad26.CoV2.S or BNT162b2 with Omicron BA.1 breakthrough infection and the unvaccinated group infected with Omicron BA.1 only. The vaccinated group was able to mount a better response over time to Omicron BA.1 in comparison to the unvaccinated group (13-fold versus 6-fold change, respectively). Unvaccinated participants displayed a lower neutralization capacity against ancestral SARS-CoV-2, Beta, Delta, and Omicron BA.2.This indicated that hybrid immunity was effective at eliciting cross-protection. .

Article

Omicron infection enhances Delta antibody immunity in vaccinated persons

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The extent to which Omicron infection[1–9], with or without previous vaccination, elicits protection against the previously dominant Delta (B.1.617.2) variant is unclear. Here we measured the neutralization capacity against variants of severe acute respiratory syndrome coronavirus 2 in 39 individuals in South Africa infected with the Omicron sublineage BA.1 starting at a median of 6 (interquartile range 3–9) days post symptom onset and continuing until last follow-up sample available, a median of 23 (interquartile range 19–27) days post symptoms to allow BA.1-elicited neutralizing immunity time to develop. Fifteen participants were vaccinated with Pfizer's BNT162b2 or Johnson & Johnson's Ad26.CoV2.S and had BA.1 breakthrough infections, and 24 were unvaccinated. BA.1 neutralization increased from a geometric mean 50% focus reduction neutralization test titre of 42 at enrolment to 575 at the last follow-up time point (13.6-fold) in vaccinated participants and from 46 to 272 (6.0-fold) in unvaccinated participants. Delta virus neutralization also increased, from 192 to 1,091 (5.7-fold) in vaccinated participants and from 28 to 91 (3.0-fold) in unvaccinated participants. At the last time point, unvaccinated individuals infected with BA.1 had low absolute levels of neutralization for the non-BA.1 viruses and 2.2-fold lower BA.1 neutralization, 12.0-fold lower Delta neutralization, 9.6-fold lower Beta variant neutralization, 17.9-fold lower ancestral virus neutralization and 4.8-fold lower Omicron sublineage BA.2 neutralization relative to vaccinated individuals infected with BA.1. These results indicate that hybrid immunity formed by vaccination and Omicron BA.1 infection should be protective against Delta and other variants. By contrast, infection with Omicron BA.1 alone offers limited cross-protection despite moderate enhancement.

The Omicron variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first identified in November 2021 in South Africa and Botswana[10], has been shown by us[1] and others[2–9] to have extensive but incomplete escape from neutralizing immunity elicited by vaccines and previous infection, with boosted individuals showing better neutralization. In South Africa, Omicron infections led to a lower incidence of severe disease relative to other variants[11,12], although this can be at least partly explained by pre-existing immunity[13]. The first Omicron sublineage to appear was BA.1, which was supplanted by the BA.2 sublineage in many countries[14].

How Omicron BA.1 infection will interact with vaccination to protect against the previously dominant Delta variant, emerging variants such

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as BA.2 and other variants is still unclear. We isolated live Omicron BA.1, Omicron BA.2, ancestral, Beta and Delta viruses and neutralized viruses with plasma from participants enrolled and longitudinally sampled during the Omicron BA.1 infection wave in South Africa, with all participants having a confirmed diagnosis of SARS-CoV-2 by quantitative PCR. To quantify neutralization, we used a live virus neutralization assay and calculated the focus reduction neutralization test ($FRNT_{50}$) titre, the inverse of the plasma dilution required for 50% neutralization, as measured by the reduction in the number of infection foci. We enrolled 41 participants who reported symptoms from late November 2021 to January 2022. We successfully sequenced the infecting virus in 26 participants, and all sequences corresponded to Omicron BA.1 (Extended Data Table 1). Two participants had advanced human immunodeficiency virus (HIV) disease on the basis of a low CD4 count (<200 cells μl^{-1} throughout the study) and unsuppressed HIV infection, and we excluded these participants because of our previous data showing an atypical response to SARS-CoV-2 in advanced HIV disease[15]. Extended Data Table 2 summarizes the characteristics of the remaining 39 participants.

Of the 39 participants, 27 were admitted to hospital because of coronavirus disease 2019 symptoms. Seven required supplemental oxygen and one died. Fifteen participants were vaccinated and had a breakthrough Omicron BA.1 infection. The median time post vaccination was 139 days (interquartile range (IQR) 120–178), a time interval that would predict considerable waning of the vaccine-elicited immune response[16], which may have contributed to the breakthrough infections. Eight participants were vaccinated with two doses of Pfizer's BNT162b2 and seven were vaccinated with Johnson & Johnson's Ad26.CoV2.S (six with a single dose and one with two doses; Extended Data Table 1). The length of hospital stay was shorter in the vaccinated (3.5 days) relative to unvaccinated (8 days; Extended Data Table 2) participants. Three participants self-reported having a previous SARS-CoV-2 infection (Extended Data Table 1).

Participants were sampled at enrolment at a median of 6 days (IQR 3–9 days) after symptom onset, and again at weekly follow-up visits that were attended as practicable because of the Christmas holidays in South Africa. The last follow-up visit was a median of 23 days (IQR 19–27 days) post-symptom onset (Extended Data Table 1). Examining neutralization at all available time points per study participant showed that neutralization of the Omicron BA.1 variant increased substantially in most participants from enrolment to the time of the last follow-up (Extended Data Fig. 1), consistent with developing a neutralizing antibody response to Omicron BA.1 infection. We therefore analysed neutralization at enrolment (baseline for the study) and the last follow-up visit to quantify the increase in neutralization capacity after Omicron infection.

We observed that Omicron BA.1 neutralization increased in vaccinated individuals from a low geometric mean titre (GMT) $FRNT_{50}$ of 42 at the enrolment visit to 575 at the last follow-up visit about 2 to 3 weeks later, a 13.6-fold change (95% CI confidence interval (CI) 3.7–50.2; Fig. 1a). The samples from unvaccinated participants at the study baseline neutralized Omicron BA.1 at a similar starting level of 46 and reached a final level of 272 at the last follow-up, a 6.0-fold increase (95% CI 2.2–16.1; Fig. 1b). Neutralization of the Delta virus also increased during this period. At enrolment, neutralization capacity against the Delta virus was 192 and reached a final level of 1,091 at the last follow-up visit in vaccinated participants, a 5.7-fold increase (95% CI 1.7–18.4; Fig. 1c). Unvaccinated participants had lower Delta neutralization at baseline with Delta virus $FRNT_{50}$ = 28, and reached $FRNT_{50}$ = 91, a 3.2-fold increase (95% CI 1.3–8.1; Fig. 1d).

We next compared Omicron BA.1 to Omicron BA.2, Delta, Beta (ref. [17]) and ancestral virus neutralization at the last available follow-up visit in three sets of paired experiments, each comparing Omicron BA.2, Delta or ancestral and Beta virus neutralization to Omicron BA.1 neutralization. The range of Omicron BA.1 neutralization shown in Fig. 2a

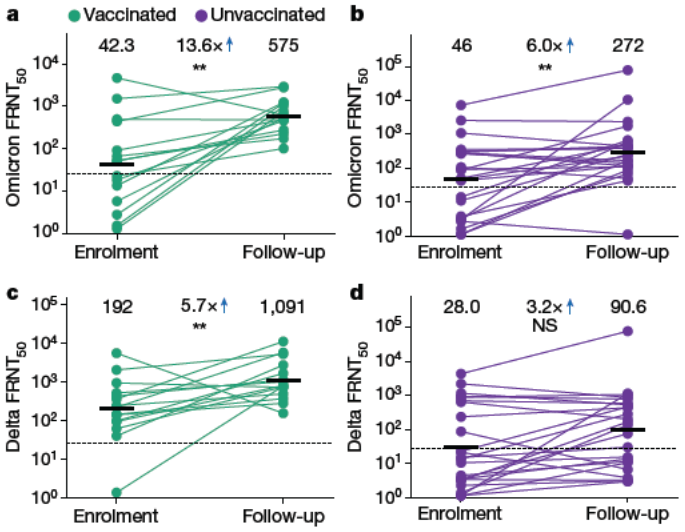

Fig. 1 | Enhancement of Delta neutralization by Omicron infection. **a**,**b**, Neutralization of the Omicron BA.1 virus over time for $n = 15$ vaccinated (**a**) and $n = 24$ unvaccinated (**b**) participants infected with Omicron BA.1. **c**,**d**, Neutralization of the Delta virus over time for the same vaccinated ($n = 15$; **c**) and unvaccinated ($n = 24$; **d**) participants as in **a**,**b**. For each participant, the sample collected at the initial enrolment visit (median 6 days post symptom onset) was compared with that collected at the last follow-up visit (median 23 days post symptom onset). The neutralization capacity per participant was determined in two independent experiments, and the numbers and horizontal bars are GMTs over all participants per group of the reciprocal plasma dilution ($FRNT_{50}$) resulting in 50% neutralization. The fold change was calculated by dividing the GMT from the follow-up by the GMT from the enrolment visit. The dashed line is the most concentrated plasma tested. The *P* values were determined by a left-sided Wilcoxon rank sum test measuring the significance of the increase; $^{**}P = 0.01–0.001$; NS, not significant. The exact *P* values are 0.0012 (**a**), 0.0081 (**b**), 0.0021 (**c**) and 0.11 (**d**).

for different experiments ($FRNT_{50}$ = 516 to 646 for vaccinated samples and 266 to 271 for unvaccinated samples) is the result of experimental variation. BA.2 neutralization was moderately and not significantly lower relative to BA.1 neutralization in both vaccinated and unvaccinated participants. Testing only participants with sequence-confirmed Omicron BA.1 infection gave a similar result (Extended Data Fig. 2). The trend for the other variants and the ancestral virus was that neutralization was higher relative to Omicron BA.1 in vaccinated participants but lower relative to Omicron BA.1 in unvaccinated participants, although the differences were mostly not significant (Fig. 2a). As a result of the relatively moderate fold change, higher participant numbers would probably be required to make the trends statistically significant.

The comparison of the other variants to Omicron BA.1 within the vaccinated or unvaccinated group does not indicate the differences in neutralization capacity elicited by Omicron BA.1 between the vaccinated and unvaccinated participants. We therefore compared neutralization of each variant between the vaccinated and unvaccinated groups at the last time point directly (Fig. 2b). The smallest difference between vaccinated and unvaccinated participants was in neutralization of Omicron BA.1, the infecting variant, with the vaccinated participants showing 2.2-fold higher neutralization. For the other variants, neutralization was higher in vaccinated participants by a factor of 4.8-fold for Omicron BA.2, 9.6-fold for Beta, 12.0-fold for Delta and 17.9-fold for ancestral (Fig. 2b). All differences were significant, and the 95% CIs for the GMT $FRNT_{50}$ of vaccinated and unvaccinated participants did not overlap for BA.2, Beta, Delta or ancestral virus neutralization (Fig. 2b). For the unvaccinated participants, the absolute neutralization capacity against the BA.2, Beta, Delta and ancestral viruses was low[18], with GMT $FRNT_{50}$ being about or below $FRNT_{50}$ = 100 (Fig. 2b).

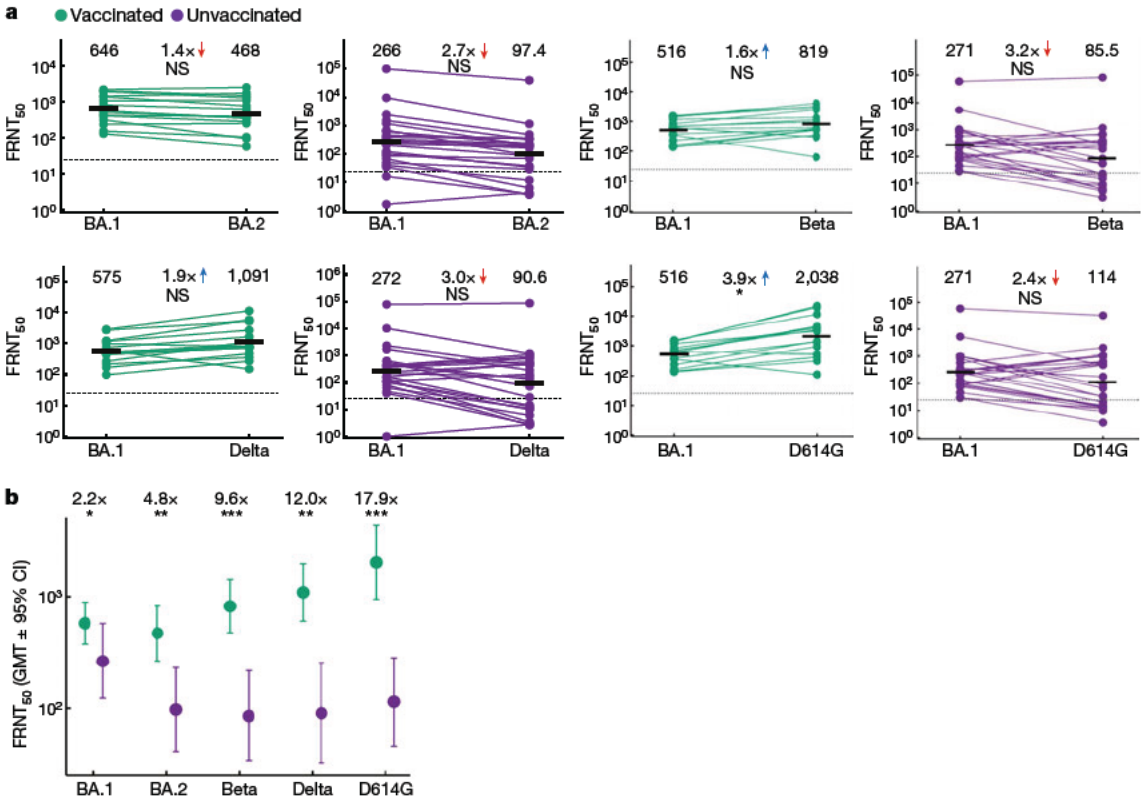

Fig. 2 | Gap in neutralizing immunity between vaccinated and unvaccinated participants infected with Omicron BA.1. a, Neutralization of Omicron BA.2, Beta, Delta and ancestral (with the D614G substitution) viruses compared to the Omicron BA.1 virus at the last available follow-up time point in $n = 15$ vaccinated or $n = 24$ unvaccinated participants infected with Omicron BA.1. The neutralization capacity per participant was determined in two independent experiments, and the numbers and horizontal bars are GMT $FRNT_{50}$. The fold change was calculated by dividing the larger by the smaller GMT. The dashed line is the most concentrated plasma tested. The *P* values were determined by a two-sided Wilcoxon rank sum test; $*P = 0.05–0.01$; NS, not significant. The exact *P* values (vaccinated/unvaccinated) are: 0.22/0.087 for BA.2, 0.36/0.071 for Beta, 0.15/0.25 for Delta and 0.014/0.20 for ancestral. **b**, Comparison of the neutralization capacity against the Omicron BA.1, Omicron BA.2, Beta, Delta and ancestral (D614G) viruses in vaccinated ($n = 15$) versus unvaccinated ($n = 24$) participants infected with Omicron BA.1. The neutralization capacity per participant was determined in two independent experiments for all strains except for Omicron BA.1, for which six experiments were available and were used in the calculation. The points represent GMT $FRNT_{50}$ per group and the error bars are GMT 95% CIs. The *P* values were determined by a two-sided Wilcoxon rank sum test; $*P = 0.05–0.01$; $**P = 0.01–0.001$; $***P = 0.001–0.0001$. The exact *P* values are 0.025 (BA.1), 0.0026 (BA.2), 4.1×10^{-4} (Beta), 0.0012 (Delta) and 3.3×10^{-4} (ancestral).

We also tested neutralization of Omicron BA.1 by Delta-variant-elicited immunity. We collected 18 plasma samples from 14 participants (including pre-vaccination and post-vaccination samples from 4 participants) previously infected in the Delta variant wave in South Africa, 8 of whom were vaccinated either before or after infection (Extended Data Table 3). Confirming previously reported results[19], we observed similar escape of Omicron BA.1 from Delta-elicited immunity across all samples tested, manifested as a 22.5-fold decrease (95% CI 14.4–35.0) in Omicron BA.1 neutralization compared to Delta virus neutralization (Fig. 3).

The large fold drop in Delta-infection-elicited neutralization capacity against Omicron BA.1 contrasts with the moderate and nonsignificant fold drops, or even fold increases, in neutralization of other variants relative to Omicron BA.1 in individuals infected with Omicron BA.1. However, in unvaccinated individuals, even though fold drops in neutralization were moderate and nonsignificant, the absolute levels of neutralization of the other variants, and of Omicron BA.1 itself, were low and on a similar scale to the cross-neutralization capacity against Omicron in Delta-infection-elicited immunity. This is consistent with other recently reported results[20], and possibly indicates that Omicron is poorly immunogenic. In agreement with recent reports[21,22], our observations show moderately and nonsignificantly lower neutralization of BA.2 by BA.1-elicited immunity. The results explain epidemiological observations showing that Omicron BA.2 reinfection is relatively rare soon after Omicron BA.1 infection[23,24].

Our results may be supportive of a scenario in which hybrid immunity formed by Omicron infection combined with vaccination protects as well or better against reinfection with variants such as Delta relative to reinfection with Omicron itself. By contrast, unvaccinated participants infected with Omicron BA.1 only, have low neutralization capacity against the Omicron BA.2, Beta, Delta and ancestral viruses.

Limitations of this study include heterogeneity in participant immune history, including two vaccination types and one boost. On the basis of the high seroprevalence observed in South Africa[25,26], some participants may also have had unreported previous infection. However, including two vaccine types did not mask the differences between vaccinated and unvaccinated participants, and the low levels of neutralization in unvaccinated participants against the ancestral, Beta and Delta viruses (the dominant strains in the preceding South African infection waves) support the notion that these participants were either not previously infected, or that immunity has waned completely. Participants were also mostly hospitalized, which may not be typical of Omicron infection[13,27]. Increased disease severity has been shown to lead to higher anti-SARS-CoV-2 antibody titres[28]. This should help in the detection of the neutralization response, but whether it would affect the trend we observed is unclear. Omicron infection is unlikely benign to the extent that hospitalization is an outlier outcome: in the USA, the number of individuals with coronavirus disease 2019 who died in the Omicron wave was similar to the number who died in the Delta

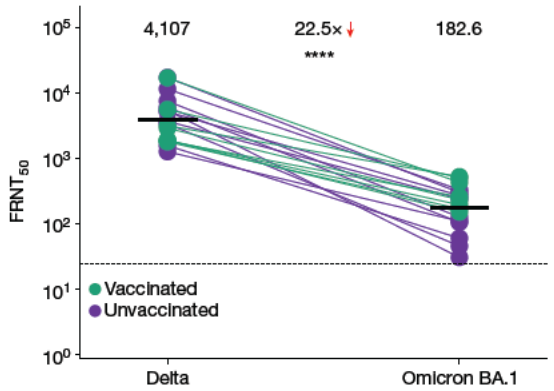

Fig. 3 | Escape of Omicron virus from Delta-infection-elicited immunity. Neutralization of Delta compared to the Omicron BA.1 virus by Delta-infection-elicited plasma immunity in vaccinated and unvaccinated participants. A total of 18 samples were tested from $n = 14$ participants infected during the Delta infection wave in South Africa. The neutralization capacity per participant was determined in two independent experiments, and the numbers and horizontal bars are GMT $FRNT_{50}$. The fold change was calculated by dividing the larger by the smaller GMT. The dashed line is the most concentrated plasma tested. $^{****}P = 3.2 \times 10^{-7}$ as determined by a two-sided Wilcoxon rank sum test.

wave[27]. Neutralizing immunity may have increased further in some participants had we sampled later: the neutralizing capacity did not plateau at the last time point in 8 of the 24 (33%) unvaccinated participants (participants 10, 14, 21, 27, 30, 31, 34 and 38; Extended Data Fig. 1) and 6 of the 15 (40%) vaccinated participants (participants 4, 6, 15, 16, 25 and 26). Therefore, the temporal dynamics give no clear indication that the immunity in the unvaccinated participants was delayed and would have reached similar levels to that of vaccinated participants if sampled later. However, the consequences of waning immunity several months post Omicron infection should be investigated.

The gap in immunity between unvaccinated individuals infected with Omicron BA.1 and vaccinated individuals with BA.1 breakthrough infection is concerning. Especially as immunity wanes, unvaccinated individuals post Omicron infection are likely to have poor cross-protection against existing and possibly emerging SARS-CoV-2 variants, despite acquiring some neutralizing immunity to the infecting Omicron BA.1 sub-lineage variant. The implication may be that Omicron BA.1 infection alone is not sufficient for protection and vaccination should be administered even in areas with a high prevalence of Omicron infection to protect against other variants.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-022-04830-x.

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Methods

Informed consent and ethics

Blood samples and the Delta isolate were obtained after written informed consent from adults with PCR-confirmed SARS-CoV-2 infection who were enrolled in a prospective cohort study at the Africa Health Research Institute approved by the Biomedical Research Ethics Committee at the University of KwaZulu-Natal (reference BREC/00001275/2020). Omicron BA.1 was isolated from a residual swab sample for SARS-CoV-2 where isolation from the sample was approved by the University of the Witwatersrand Human Research Ethics Committee (HREC; reference M210752). The sample to isolate Omicron BA.2 was collected after written informed consent as part of the study "COVID-19 transmission and natural history in KwaZulu-Natal, South Africa: Epidemiological Investigation to Guide Prevention and Clinical Care" of the Centre for the AIDS Programme of Research in South Africa (CAPRISA) and isolation from the sample approved by the Biomedical Research Ethics Committee at the University of KwaZulu-Natal (reference BREC/00001195/2020, BREC/00003106/2021).

Reagent availability

Virus isolates and the cell line are available from the corresponding author. A Biosafety Level 3 facility is required for laboratories receiving live SARS-CoV-2.

Whole-genome sequencing, genome assembly and phylogenetic analysis

RNA was extracted on an automated Chemagic 360 instrument, using the CMG-1049 kit (Perkin Elmer). The RNA was stored at −80 °C before use. Libraries for whole-genome sequencing were prepared using either the Oxford Nanopore Midnight protocol with Rapid Barcoding or the Illumina COVIDseq Assay. For the Illumina COVIDseq assay, the libraries were prepared according to the manufacturer's protocol. Briefly, amplicons were tagmented, followed by indexing using the Nextera UD Indexes Set A. Sequencing libraries were pooled, normalized to 4 nM and denatured with 0.2 N sodium acetate. An 8 pM sample library was spiked with 1% PhiX (PhiX Control v3 adaptor-ligated library used as a control). We sequenced libraries on a 500-cycle v2 MiSeq Reagent Kit on the Illumina MiSeq instrument (Illumina). On the Illumina NextSeq 550 instrument, sequencing was performed using the Illumina COVIDSeq protocol (Illumina), an amplicon-based next-generation sequencing approach. The first-strand synthesis was carried using random hexamer primers from Illumina, and the synthesized cDNA underwent two separate multiplex PCR reactions. The pooled PCR-amplified products were processed for tagmentation and adaptor ligation using IDT for Illumina Nextera UD Indexes. Further enrichment and cleanup was performed as per protocols provided by the manufacturer (Illumina). Pooled samples were quantified using a Qubit 3.0 or 4.0 fluorometer (Invitrogen) through the Qubit dsDNA High Sensitivity Assay according to the manufacturer's instructions. The fragment sizes were analysed using TapeStation 4200 (Invitrogen). The pooled libraries were further normalized to 4 nM concentration and 25 µl of each normalized pool containing unique index adaptor sets was combined in a new tube. The final library pool was denatured and neutralized with 0.2 N sodium hydroxide and 200 mM Tris-HCl (pH 7), respectively. A 1.5 pM sample library was spiked with 2% PhiX. Libraries were loaded onto a 300-cycle NextSeq 500/550 HighOutput Kit v2 and run on the Illumina NextSeq 550 instrument (Illumina). For Oxford Nanopore sequencing, the Midnight primer kit was used: cDNA synthesis was performed on the extracted RNA using LunaScript RT mastermix (New England BioLabs) followed by gene-specific multiplex PCR using the Midnight Primer pools that produce 1,200-base-pair amplicons that overlap to cover the 30-kb SARS-CoV-2 genome. Amplicons from each pool were pooled and used neat for barcoding with the Oxford Nanopore Rapid Barcoding kit as per the manufacturer's protocol. Barcoded samples were pooled and bead purified. After the bead cleanup, the library was loaded on a prepared R9.4.1 flow cell. A GridION X5 or MinION sequencing run was initiated using MinKNOW software with the base-call setting switched off. We assembled paired-end and nanopore.fastq reads using Genome Detective 1.132 (https://www.genomedetective.com), which was updated for the accurate assembly and variant calling of tiled primer amplicon Illumina or Oxford Nanopore reads, and the Coronavirus Typing Tool. For Illumina assembly, the GATK HaploTypeCaller –min-pruning 0 argument was added to increase mutation calling sensitivity near sequencing gaps. For Nanopore, low-coverage regions with poor alignment quality (<85% variant homogeneity) near sequencing/amplicon ends were masked to be robust against primer drop-out experienced in the spike gene, and the sensitivity for detecting short inserts using a region-local global alignment of reads was increased. In addition, we also used the wf_artic (ARTIC SARS-CoV-2) pipeline as built using the nextflow workflow framework. In some instances, mutations were confirmed visually with .bam files using Geneious software V2020.1.2 (Biomatters). The reference genome used throughout the assembly process was NC_045512.2 (numbering equivalent to MN908947.3). For lineage classification, we used the widespread dynamic lineage classification method from the Phylogenetic Assignment of Named Global Outbreak Lineages software suite (https://github.com/hCoV-2019/pangolin).

Cells

Vero E6 cells (originally ATCC CRL-1586, obtained from Cellonex in South Africa) were propagated in complete growth medium consisting of Dulbecco's modified Eagle medium with 10% fetal bovine serum (Hyclone) containing 10 mM HEPES, 1 mM sodium pyruvate, 2 mM L-glutamine and 0.1 mM nonessential amino acids (Sigma-Aldrich). Vero E6 cells were passaged every 3–4 days. The H1299-E3 cell line (H1299 originally from ATCC as CRL-5803) was propagated in growth medium consisting of complete Roswell Park Memorial Institute 1640 medium with 10% fetal bovine serum containing 10 mM HEPES, 1 mM sodium pyruvate, 2 mM L-glutamine and 0.1 mM nonessential amino acids. Cells were passaged every second day. The H1299-E3 (H1299-ACE2, clone E3) cell line was derived from H1299 as described in our previous work[1,17]. Cell lines were not authenticated. Cell lines tested negative for mycoplasma contamination.

Virus expansion

All work with live virus was performed in Biosafety Level 3 containment using protocols for SARS-CoV-2 approved by the Africa Health Research Institute Biosafety Committee. ACE2-expressing H1299-E3 cells were seeded at 4.5×10^5 cells in a 6-well plate well and incubated for 18–20 h. After one Dulbecco's phosphate-buffered saline (PBS) wash, the subconfluent cell monolayer was inoculated with 500 µl universal transport medium diluted 1:1 with growth medium filtered through a 0.45-µm filter. Cells were incubated for 1 h. Wells were then filled with 3 ml complete growth medium. After 4 days of infection (completion of passage 1 (P1)), cells were trypsinized, centrifuged at 300 RCF for 3 min and resuspended in 4 ml growth medium. All cells from the P1 infection were added to Vero E6 cells that had been seeded at 2×10^5 cells ml^{-1}, 20 ml total, 18–20 h earlier in a T75 flask for cell-to-cell infection. The coculture of ACE2-expressing H1299-E3 and Vero E6 cells was incubated for 1 h, and the flask was filled with 20 ml of complete growth medium and incubated for 4 days. The viral supernatant from this culture (passage 2 (P2) stock) was used for experiments.

Live virus neutralization assay

H1299-E3 cells were plated in a 96-well plate (Corning) at 30,000 cells per well 1 day pre-infection. Plasma was separated from EDTA-anticoagulated blood by centrifugation at 500 RCF for 10 min and stored at −80 °C. Aliquots of plasma samples were heat-inactivated at 56 °C for 30 min and clarified by centrifugation at 10,000 RCF for

5 min. Virus stocks were used at approximately 50–100 focus-forming units per microwell and added to diluted plasma. Antibody–virus mixtures were incubated for 1 h at 37 °C, 5% CO_2. Cells were infected with 100 µl of the virus–antibody mixtures for 1 h, and then 100 µl of a 1× Roswell Park Memorial Institute 1640 (Sigma-Aldrich, R6504), 1.5% carboxymethylcellulose (Sigma-Aldrich, C4888) overlay was added without removing the inoculum. Cells were fixed 18 h after infection using 4% paraformaldehyde (Sigma-Aldrich) for 20 min. Foci were stained with a rabbit anti-spike monoclonal antibody (BS-R2B12, GenScript A02058) at 0.5 µg ml^{-1} in a permeabilization buffer containing 0.1% saponin (Sigma-Aldrich), 0.1% BSA (Sigma-Aldrich) and 0.05% Tween-20 (Sigma-Aldrich) in PBS. Plates were incubated with primary antibody overnight at 4 °C, and then washed with wash buffer containing 0.05% Tween-20 in PBS. Secondary goat anti-rabbit antibody conjugated to horseradish peroxidase (Abcam ab205718) was added at 1 µg ml^{-1} and incubated for 2 h at room temperature with shaking. TrueBlue peroxidase substrate (SeraCare 5510-0030) was then added at 50 µl per well and incubated for 20 min at room temperature. Plates were imaged in an ImmunoSpot Ultra-V S6-02-6140 Analyzer ELISPOT instrument with BioSpot Professional built-in image analysis (Cellular Technology Limited).

Statistics and fitting

Statistical methods were not used to predetermine sample size, and blinding and randomization were not used. All statistics and fitting were performed using custom code in MATLAB v.2019b. Neutralization data were fitted to:

$$\mathrm{Tx} = 1/1 + (D/\mathrm{ID}_{50}).$$

Here Tx is the number of foci normalized to the number of foci in the absence of plasma on the same plate at dilution D, and ID_{50} is the plasma dilution giving 50% neutralization. $FRNT_{50} = 1/ID_{50}$. Values of $FRNT_{50} < 1$ are set to 1 (undiluted), the lowest measurable value. We note that the most concentrated plasma dilution was 1:25 and therefore $FRNT_{50} < 25$ data were extrapolated. To calculate CIs, $FRNT_{50}$ or fold change in $FRNT_{50}$ per participant was log transformed and the arithmetic mean plus 2 s.d. and the arithmetic mean minus 2 s.d. were calculated for the log-transformed values. These were exponentiated to obtain the upper and lower 95% CIs on the geometric mean $FRNT_{50}$ or the fold change in $FRNT_{50}$ geometric means.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Sequences of outgrown Omicron sublineages have been deposited in GISAID (https://www.gisaid.org/) with accessions EPI_ISL_7886688 (Omicron BA.1), EPI_ISL_9082893 (Omicron BA.2) and EPI_ISL_602626.1 (ancestral/D614G). Delta (EPI_ISL_3118687) and Beta (EPI_ISL_678615) isolates have been described previously[15]. Raw images of the data are available upon reasonable request. Source data are provided with this paper.

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Author contributions A.Sigal., K.K. and F.K. conceived the study and designed the study and experiments. A.v.G., Q.A.K., S.S.A.K., G.L., A.Sivro and N.S. identified and provided virus samples. M.-Y.S.M., F.K., B.I.G., M.B., K.K., T.N., M.M., N. Mthabela, Z.M., N.N., Y.M., N. Mbatha, N. Manickchund, N. Magula, Z.J., K.R. and Y.G. set up and managed the cohort and cohort data. K.K., Z.J., K.R., S.C., H.T., J.E.S., Y.G., J.G., Y. Ramphal, A.B.M.K., D.A. and J.N.B. performed experiments and sequence analysis with input from A.Sigal, T.d.O., R.J.L. and J.N.B., A.Sigal, K.K., F.K., R.M. and Y. Rosenberg interpreted data with input from M.-Y.S.M., G.G., S.S.A.K., W.H., T.d.O., N. Magula, R.J.L. and P.L.M., A.Sigal, K.K., G.L., F.K. and M.B. prepared the manuscript with input from all authors.

Competing interests The authors declare no competing interests.

Additional information

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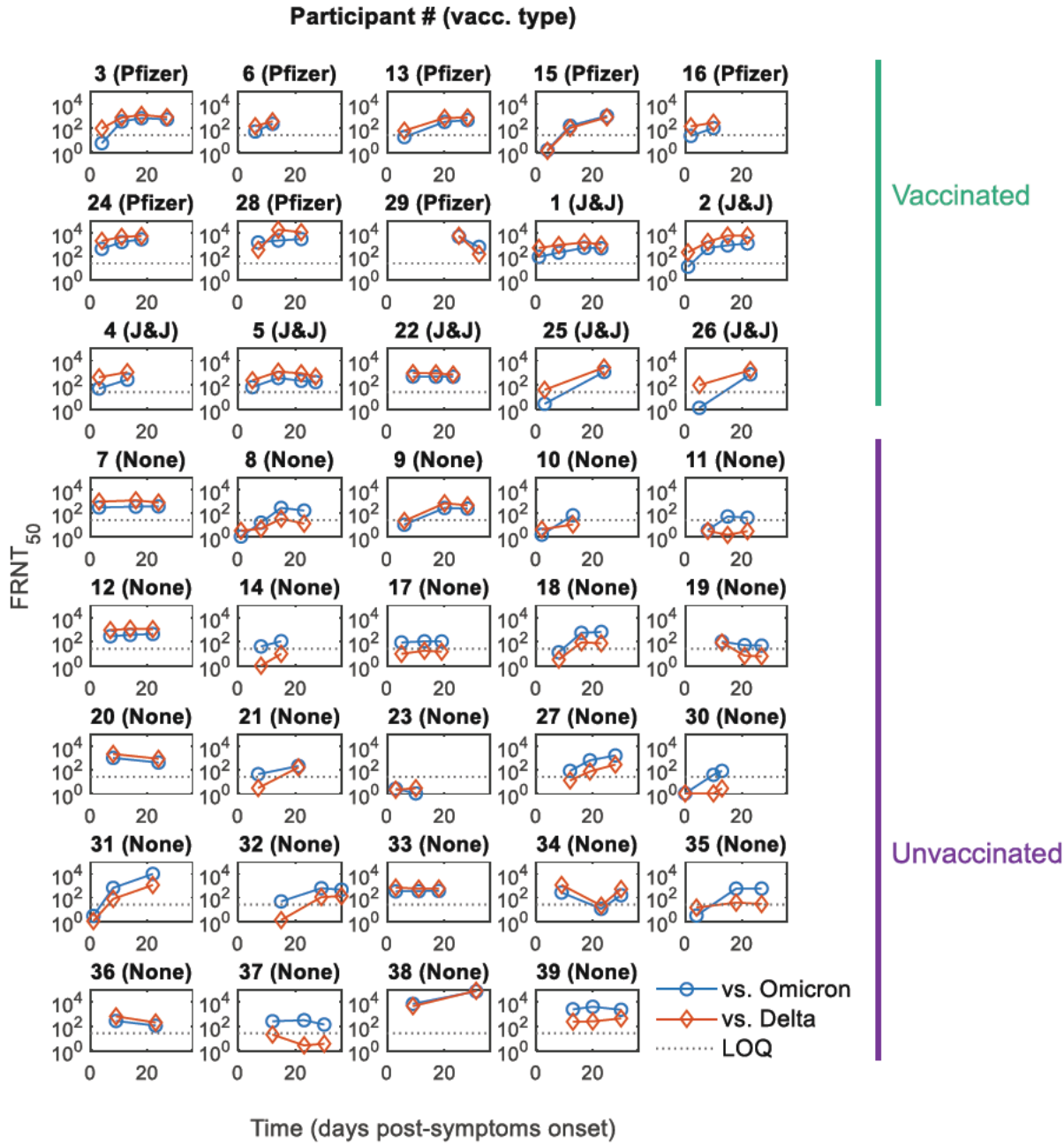
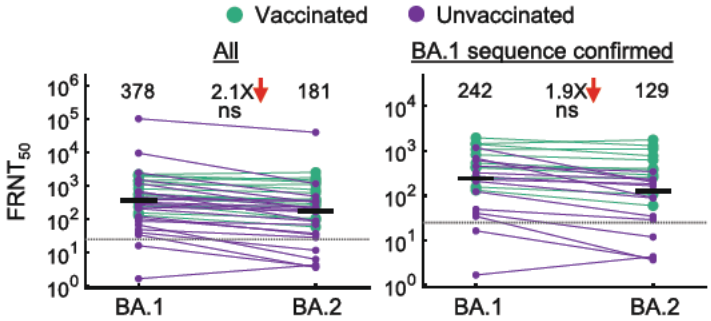


Extended Data Fig. 1 | Longitudinal Omicron/BA.1 and Delta neutralization capacity in Omicron/BA.1 infected participants. Neutralization of Omicron (blue) and Delta (red) at all study visits. Participant number is as in Extended Data Table 1. Top three rows are participants vaccinated with Pfizer BNT162b2 (n = 8) or Johnson and Johnson Ad26.CoV2.S (n = 7) and bottom five rows are unvaccinated participants (n = 24). X-axis is the time post-symptom onset when sample was collected, and y-axis is neutralization as $FRNT_{50}$. Dashed line is the most concentrated plasma tested (LOQ, limit of quantification below which $FRNT_{50}$ values are extrapolated). All participants recovered except participant 29, who died.



Extended Data Fig. 2 | Fold-drop in BA.2 versus BA.1 neutralization in all and sequence confirmed samples. Neutralization of Omicron BA.2 compared to BA.1 in participants described in Extended Data Table 1, excluding participant 14 for technical reasons and participants 40 and 41 because of advanced HIV disease. Left panel shows neutralization capacity in all n = 38 participants and right panel shows neutralization capacity for n = 25 participants where infection was successfully sequenced and determined to be BA.1. Dashed line is the most concentrated plasma tested. p-values were 0.077 for all and 0.15 for BA.1 sequence confirmed participants as determined by a two-sided Wilcoxon rank sum test. ns, not significant.

Extended Data Table 1 | Characteristics of Omicron/BA.1 infected participants

#	Age	Sex	Vacc. type	Date of vacc.	Vacc. to enrol. (days)	Date symp. onset	Ct at enrol.	Symp. to enrol. (days)	Symp. to last follow-up (days)	Max CD4	Sub-lineage	Seq. GISAID ID	Supp. O_2	Hosp.
1	30-39	M	AD26.COV2	MAR-2021	274	DEC-2021*	24.9	1	23	1071	BA.1	EPI_ISL_9967759	No	No
2	30-39	M	AD26.COV2*	NOV-2021	14	NOV-2021	14.5	1	22	789	BA.1	EPI_ISL_9967761	No	No
3	50-59	F	BNT162b2	JUL-2021	138	DEC-2021	16.8	4	27	777	BA.1	EPI_ISL_8604915	No	No
4	30-39	F	AD26.COV2	MAY-2021	210	DEC-2021	30.7	3	13	1169	BA.1	EPI_ISL_8604910	No	No
5	20-29	F	AD26.COV2	SEP-2021	89	DEC-2021	23.9	5	27	1220	BA.1	EPI_ISL_9967760	No	Yes
6	10-19	F	BNT162b2	JUL-2021	157	DEC-2021	23.1	6	12	732	BA.1	EPI_ISL_8604906	No	Yes
7	20-29	F				NOV-2021	UND	3	24	712	N/A	N/A	No	Yes
8	30-39	M				DEC-2021	18.2	1	23	847	BA.1	EPI ISL 8604919	No	Yes
9	40-49	F				DEC-2021	32.3	6	28	1032	BA.1	EPI_ISL_8604901	No	Yes
10	20-29	M				DEC-2021	30.4	2	13	1197	BA.1	EPI ISL 8604908	No	Yes
11	20-29	F				DEC-2021	28.3	8	22	863	BA.1	EPI_ISL_8604913	No	No
12	20-29	F				DEC-2021*	UND	7	22	1259	BA.1	EPI_ISL_8604912	No	Yes
13	30-39	M	BNT162b2	JUL-2021	129	NOV-2021	31.6	6	28	1069	BA.1	EPI_ISL_8604916	No	Yes
14	20-29	M				NOV-2021	30.8	8	15	1225	N/A	N/A	No	Yes
15	60-69	F	BNT162b2	JUL-2021	139	DEC-2021	24.6	4	25	345	BA.1	EPI_ISL_8604920	No	Yes
16	60-69	M	BNT162b2	DEC-2021	15	DEC-2021	24.6	2	10	904	BA.1	EPI_ISL_8578311	No	No
17	30-39	M				DEC-2021	37.0	5	19	1008	BA.1	EPI_ISL_8604923	No	No
18	60-69	F				DEC-2021	26.8	8	23	1111	BA.1	EPI_ISL_8578312	Yes	Yes
19	30-39	M				DEC-2021*	30.7	13	27	1077	BA.1	EPI_ISL_8604924	No	Yes
20	20-29	F				DEC-2021	35.9	8	24	533	BA.1	EPI_ISL_8604911	No	Yes
21	20-29	M				DEC-2021	29.1	7	21	225	BA.1	EPI_ISL_8604922	No	No
22	30-39	F	AD26.COV2	AUG-2021	120	DEC-2021	33.4	9	23	777	BA.1	EPI_ISL_8693907	No	Yes
23	20-29	F				DEC-2021	35.8	3	10	1167	BA.1	EPI_ISL_8604902	No	No
24	50-59	M	BNT162b2	AUG-2021	128	DEC-2021	36.6	4	18	605	N/A	N/A	No	Yes
25	30-39	F	AD26.COV2	APR-2021	237	DEC-2021	23.5	3	24	640	BA.1	EPI_ISL_8604914	No	No
26	50-59	F	AD26.COV2	JUL-2021	150	DEC-2021	UND	5	23	716	N/A	N/A	No	No
27	50-59	F				DEC-2021	32.4	12	28	625	N/A	N/A	Yes	Yes
28	80-89	F	BNT162b2	JUL-2021	177	JAN-2022	30.8	7	22	407	N/A	N/A	Yes	Yes
29	60-69	M	BNT162b2	JUL-2021	178	DEC-2021[$]	UND	25	32	351	N/A	N/A	Yes	Yes
30	40-49	M				DEC-2021	20.2	0	13	844	BA.1	EPI_ISL_8604909	No	No
31	30-39	F				DEC-2021	34.8	1	22	647	N/A	N/A	Yes	Yes
32	50-59	F				DEC-2021	28.2	15	36	620	BA.1	EPI ISL 8578347	No	Yes
33	20-29	F				DEC-2021	UND	3	18	902	N/A	N/A	No	Yes
34	30-39	F				DEC-2021	34.8	9	30	1363	N/A	N/A	No	Yes
35	50-59	F				DEC-2021	26.6	4	27	766	BA.1	EPI_ISL_8578342	Yes	Yes
36	20-29	F				DEC-2021	UND	9	23	1212	N/A	N/A	No	Yes
37	50-59	F				DEC-2021	UND	12	30	995	N/A	N/A	No	Yes
38	30-39	M				DEC-2021	UND	9	31	746	N/A	N/A	No	Yes
39	50-59	F				DEC-2021	UND	13	30	840	N/A	N/A	Yes	Yes
40	30-39	F				DEC-2021	22.5	5	19	61***	BA.1	EPI_ISL_8578314	Yes	Yes
41	40-49	F				NOV-2021	29.8	17	24	53***	N/A	N/A	No	Yes

Ct enrol.: qPCR cycle threshold for SARS-CoV-2 at enrollment. Symptoms to enrol.: time between symptoms onset and study enrolment. Symp. to last follow-up: time between symptoms onset and last follow-up visit. Max CD4: maximum CD4 count per microliter blood across all study visits. Supp O_2: participant required supplemental oxygen during the study. Hosp.: participant hospitalized during the study. UND: Undetectable Ct. N/A: Not available; sequencing failed, usually due to insufficient virus substrate. *Reported previous infection. **Boosted with Ad26.CoV2.S in Nov-2021. ***Participants with persistent low CD4 count and uncontrolled HIV viremia indicative of advanced HIV disease and immune suppression. Excluded from analysis. [$]Deceased.

Extended Data Table 2 | Summary characteristics of Omicron/BA.1 infected participants

	All 39	Vaccinated 15 (38%)	Unvaccinated 24 (62%)
Age	35 (27-55)	37 (32-60)	31.5 (26-49)
Female	25 (64%)	9 (60%)	16 (67%)
Vaccination to enrollment (days)	-	139 (120-178)	-
Symptom onset to enrolment (days)	6 (3-9)	4 (3-6)	7.5 (3-9)
Symptom onset to last follow-up (days)	23 (19-27)	23 (18-27)	23 (20-28)
Maximum CD4 count (cell/μL)	844 (647-1077)	777 (605-1069)	882.5 (729-1139)
Required supp. O_2	7 (18%)	2 (13%)	5 (21%)
Hospitalized	27 (69%)	8 (53%)	19 (79%)
Duration of hospitalization (days)	7 (3-11)	3.5 (2.5-14.5)	8 (3-11)

Values are median (IQR). Hospital stay calculated to last inpatient study visit.

Extended Data Table 3 | Characteristics of Delta infected participants

#	Age	Sex	Vacc. type	Date of vacc.	Vacc. to enrol. (days)	Date symp. onset	Ct at enrol.	Symp. to collection (days)	Seq. GISAID ID
1	40-49	F				JUL-2021	26	26	EPI_ISL_3722338
2	40-49	M				JUL-2021	31	23*	EPI_ISL_3722335
3	50-59	M				JUL-2021	30	31	N/A
4	50-59	M				JUN-2021	27	37	N/A
5	40-49	M				JUL-2021	35	44	N/A
6	30-39	M				JUL-2021	37	32	N/A
7	70-79	M	BNT162b2	JUN-2021	37	JUL-2021	37	15	N/A
8	60-69	F	BNT162b2	NOV-2021	14	AUG-2021	UND	116	N/A
9	40-49	F	AD26.COV	MAY-2021	117	JUL-2021	UND	31	N/A
10	50-59	F	AD26.COV	APR-2021	147	JUL-2021	UND	57	N/A
11 Pre	40-49	M				AUG-2021*	35	13*	N/A
11 Post	40-49	M	BNT162b2	OCT-2021	18	AUG-2021	UND	83	N/A
12 Pre	40-49	M				JUL-2021	23	24	EPI_ISL_3939068
12 Post	40-49	M	AD26.COV	SEP-2021	32	JUL-2021	UND	92	N/A
13 Pre	30-39	M				JUL-2021	27	24	EPI_ISL_3939088
13 Post	30-39	M	AD26.COV	SEP-2021	32	JUL-2021	UND	94	N/A
14 Pre	50-59	F				JUL-2021*	27	23*	EPI_ISL_3447779
14 Post	50-59	F	BNT162b2	OCT-2021	22	JUL-2021	UND	93	N/A

*Asymptomatic, date of diagnostic swab used instead of symptoms onset. Ct enrol: qPCR cycle threshold for SARS-CoV-2 at enrollment. UND: undetectable. Pre: sample taken pre-vaccination. Post: sample taken post-vaccination for participants with a pre-vaccination sample. N/A: not available.

CHAPTER 4: OMICRON SUB-LINEAGES BA.4/BA.5 ESCAPE FROM BA.1 INFECTION ELICITED NEUTRALIZING IMMUNITY

Three months into South Africa's fourth Wave, Omicron evolved into subvariants BA.4 (22A) and BA.5 (22B). The subvariants had changes in the spike RBD relative to Omicron BA.1 and BA.2 (Karim and Karim, 2021). Live virus was isolated and used to measure how well previous infection with BA.1 protects against these two subvariants, in the presence or absence of vaccination. In the unvaccinated group, considerable escape of BA.4 and BA.5 from BA.1 infection elicited immunity was observed. However, in the vaccinated group, a reduced fold-drop of 3.2-fold for BA.4 and 2.6 for BA.5 was observed. This indicates that hybrid immunity attenuates the escape of emerging variants from infection elicited neutralizing immunity of the previously dominant variant.

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Omicron BA.4/BA.5 escape neutralizing immunity elicited by BA.1 infection

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SARS-CoV-2 Omicron (B.1.1.529) BA.4 and BA.5 sub-lineages, first detected in South Africa, have changes relative to Omicron BA.1 including substitutions in the spike receptor binding domain. Here we isolated live BA.4 and BA.5 viruses and measured BA.4/BA.5 neutralization elicited by BA.1 infection either in the absence or presence of previous vaccination as well as from vaccination without BA.1 infection. In BA.1-infected unvaccinated individuals, neutralization relative to BA.1 declines 7.6-fold for BA.4 and 7.5-fold for BA.5. In vaccinated individuals with subsequent BA.1 infection, neutralization relative to BA.1 decreases 3.2-fold for BA.4 and 2.6-fold for BA.5. The fold-drop versus ancestral virus neutralization in this group is 4.0-fold for BA.1, 12.9-fold for BA.4, and 10.3-fold for BA.5. In contrast, BA.4/BA.5 escape is similar to BA.1 in the absence of BA.1 elicited immunity: fold-drop relative to ancestral virus neutralization is 19.8-fold for BA.1, 19.6-fold for BA.4, and 20.9-fold for BA.5. These results show considerable escape of BA.4/BA.5 from BA.1 elicited immunity which is moderated with vaccination and may indicate that BA.4/BA.5 may have the strongest selective advantage in evading neutralization relative to BA.1 in unvaccinated, BA.1 infected individuals.

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New severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants may escape neutralizing immunity elicited by previous infection and vaccination and lead to new infection waves. Therefore, the degree to which such immune escape happens with new variants needs to be measured globally in different populations. This may be particularly informative for the region where the variant was first detected, as it may indicate the selective pressures under which the new variant evolved.

The Omicron (Pango lineage B.1.1.529) initially emerged as the BA.1 sub-lineage. BA.1 was first detected by genomic surveillance in South Africa and showed extensive immune escape[1–13]. The BA.4 and BA.5 sub-lineages, which do not differ in their spike sequence from each other, were also first detected by genomic surveillance in South Africa[14]. BA.4 and BA.5 have changes relative to the BA.1 and BA.2 sub-lineages including the L452R and F486V mutations and the R493Q reversion in the spike receptor binding domain (RBD), the domain which is likely most targeted by neutralizing antibodies[15]. BA.4 and BA.5 also differ from the BA.2 sub-lineage by a deletion of spike residues 69 and 70[16]. The L452R mutation has been reported to increase SARS-CoV-2 fusogenicity and replication in cell culture[17,18]. This mutation also occurs in the Delta variant and a mutation at spike position L452 is shared with the Omicron sub-lineage BA.2.12.1, where the substitution is L452Q[16]. The F486V mutation is located at the top of the spike receptor-binding ridge that contacts the human angiotensin converting enzyme-2 (ACE2) receptor, and is associated with escape from class 1 and class 2 RBD antibodies[19]. That is, the predominant effect of this mutation is expected to be antibody escape. Interestingly, despite its predicted ability to confer escape from neutralization, this mutation was previously rarely observed[19], possibly indicating it confers a fitness disadvantage which is compensated by other mutations in the BA.4 and BA.5 Omicron sub-lineages.

Starting in March 2022, the BA.4 and BA.5 sub-lineages led to an infection wave in South Africa which has since waned (Fig. 1a, see https://www.nicd.ac.za/diseases-a-z-index/disease-index-covid-19/surveillance-reports/national-covid-19-daily-report/ for source data). Excess all-cause mortality in South Africa, which was previously strongly correlated to SARS-CoV-2 infection waves, did not show a sharp increase in the BA.4/BA.5 infection wave, although excess deaths were still present (Fig. 1a, see source data at https://www.samrc.ac.za/reports/report-weekly-deaths-south-africa). While the fraction of BA.4 and BA.5 genotypes has stabilized at about three-quarters of all infections in South Africa as this is written (Fig. 1b, all data from GISAID[20]), infections with these sub-lineages are rising elsewhere, including in the US (Fig. 1b). About half of the South African population was vaccinated when BA.4 and BA.5 were first detected (Fig. 1c). Vaccination in South Africa is currently with one of two vaccines, two doses of the Pfizer BNT162b2 or one dose of the Johnson and Johnson Ad26.CoV2.S. At the time of writing about 8 million South Africans were fully vaccinated with Ad26.CoV2.S compared to about 12 million vaccinated with BNT162b2 (https://sacoronavirus.co.za/latest-vaccine-statistics/). Boosting is available in South Africa, although it was too rare in our cohort for us to investigate.

In this work, we measure the degree of escape of the BA.4 and BA.5 sub-lineages from neutralizing immunity in people previously infected with the Omicron BA.1 in South Africa and determine the effect of vaccination on immune escape using live viral isolates. We also compare immune escape of BA.1, BA.4, and BA.5 in vaccinated individuals from South Africa not infected with BA.1.

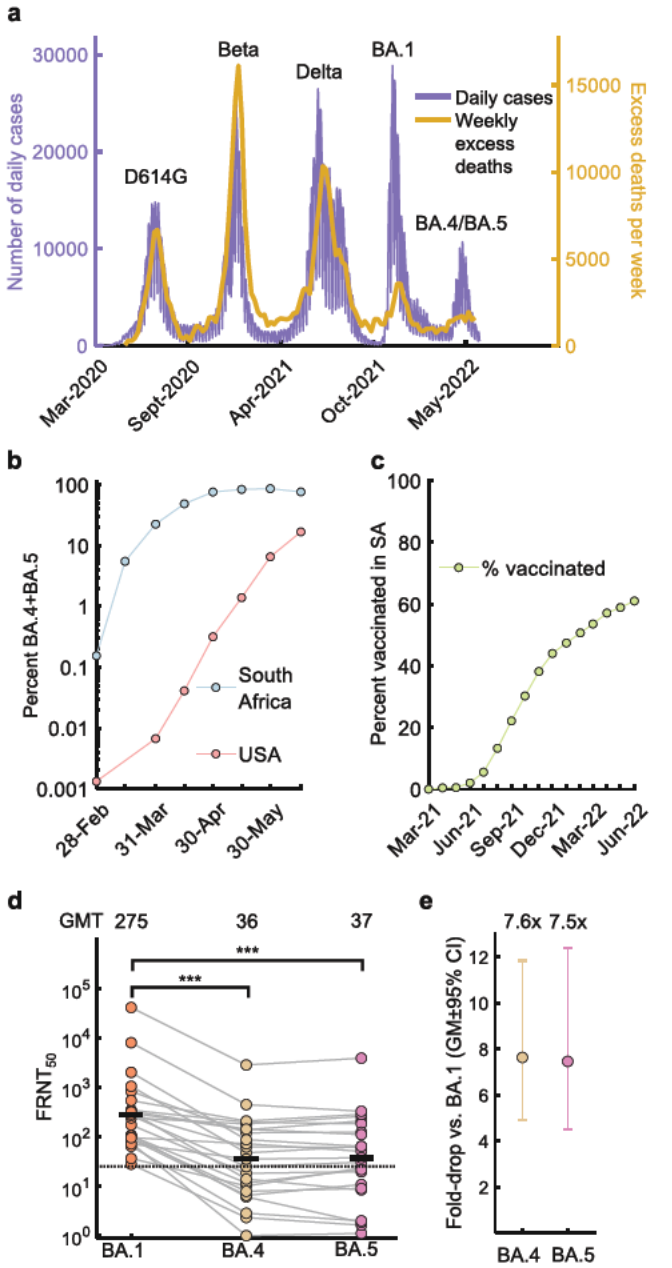

Fig. 1 | Escape of BA.4 and BA.5 from BA.1 elicited immunity in unvaccinated participants. a Daily Covid-19 cases (purple, left axis) and excess deaths (orange, right axis) in South Africa. **b** Combined fraction of BA.4 and BA.5 in South Africa and the US according to GISAID deposited sequence data. Prevalence was calculated by dividing the number of submitted BA.4 and BA.5 sequences by total submitted sequences per 2-week period starting February 15, 2022. **c** Percentage of South Africans vaccinated over time. **d** Neutralization of BA.4 and BA.5 compared to BA.1 virus by BA.1 infection elicited neutralizing immunity in $n = 24$ unvaccinated participants. Numbers are geometric mean titer (GMT) $FRNT_{50}$. Dashed line is most concentrated plasma tested. **e** Geometric mean (GM) of fold-drops in neutralization and their 95% confidence intervals for BA.4 and BA.5 relative to BA.1 calculated from (**d**). For panels (**d**) and (**e**), orange points represent BA.1, yellow BA.4, and pink BA.5. *p*-values were determined by a two-sided Wilcoxon rank sum test and represented as ***0.001-0.0001. Exact *p*-values were 4.4×10^{-4} for both BA.4 and BA.5. Source data are provided as a Source Data file.

Results

We isolated live BA.4 and BA.5 viruses from infections in South Africa to test against pre-existing immunity. This consisted of sera from unvaccinated ($n = 24$) and vaccinated ($n = 15$) people infected in the preceding infection wave which was BA.1 dominated (Fig. 1a). This cohort was previously described by us[21] and consisted of participants with mostly mild Omicron BA.1 infections who were sampled weekly from symptom onset. Samples used here were collected a median of 23 days (IQR 19 27 days) post-symptom onset, once the participants developed or increased their BA.1 neutralizing response[21]. We also

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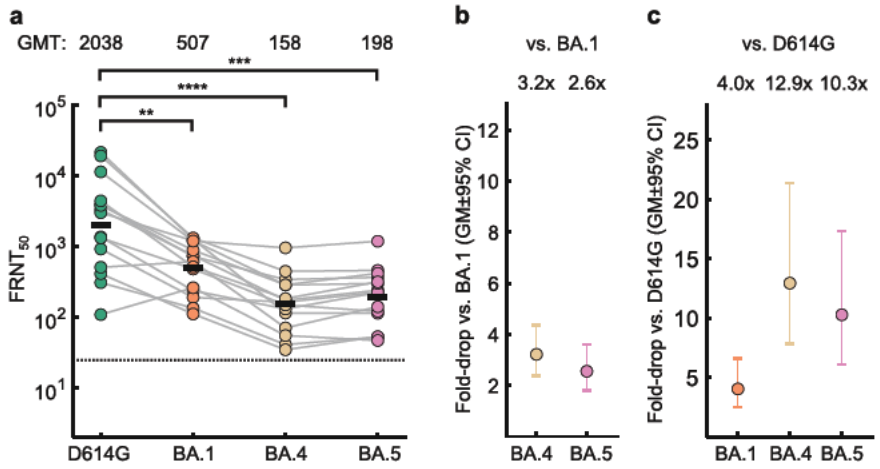

Fig. 2 | Escape of BA.4 and BA.5 from immunity elicited by vaccination combined with BA.1 breakthrough infection. a Neutralization of ancestral virus with the D614G substitution, BA.1, BA.4 and BA.5 by vaccine elicited neutralizing immunity with BA.1 breakthrough infection in $n = 15$ participants. Numbers are geometric mean titer (GMT) $FRNT_{50}$. Dashed line is most concentrated plasma tested. **b** Geometric mean (GM) of fold- drops in neutralization and their 95% confidence intervals for BA.4 and BA.5 relative to BA.1 calculated from (**a**). **c** GM of fold-drops in neutralization and their 95% confidence intervals for BA.1, BA.4 and BA.5 relative to ancestral/D614G virus calculated from (**a**). For all panels, green points are values for ancestral/D614G, orange points are BA.1, yellow points are BA.4, and pink points are BA.5. *p*-values were determined by a two-sided Wilcoxon rank sum test and represented as **0.01-0.001, ***0.001-0.0001, ****<0.0001. Exact *p*-values were 7.9×10^{-3} for BA.1, 9.7×10^{-5} for BA.4, and 1.9×10^{-4} for BA.5. Source data are provided as a Source Data file.

tested the viruses against sera from people who were vaccinated but not BA.1 infected ($n = 18$, see Supplementary Table 1 for cohort details). For study participants infected in the Omicron BA.1 infection wave, the majority (25 out of 39 infections) were confirmed Omicron/BA.1 by sequencing the infecting virus[21] (Table S1).

To quantify neutralization, we report the 50% focus reduction neutralization test value ($FRNT_{50}$), which is the inverse of the plasma dilution required for a 50% reduction in the number of infection foci relative to the no antibody control in a live virus neutralization assay[22].

We first report neutralization in the 24 unvaccinated study participants infected with BA.1. Neutralization of BA.1 was low at $FRNT_{50}$ = 275. The $FRNT_{50}$ declined to 36 for BA.4 and 37 for BA.5 neutralization (Fig. 1d), 7.5 and 7.6-fold drops, respectively relative to BA.1 neutralization (Fig. 1e).

In vaccinated participants with BA.1 breakthrough infection after vaccination, BA.4 and BA.5 neutralization dropped from 507 for BA.1 to 158 for BA.4 and 198 BA.5 (Fig. 2a). The corresponding fold-drops were 3.2 for BA.4 and 2.6 for BA.5 (Fig. 2b). Given that the vaccines were designed with ancestral SARS-CoV-2 sequence, neutralization capacity against the ancestral virus with the D614G substitution may be a second benchmark to measure escape in this group. We therefore compared the neutralization of the Omicron sub-lineages to neutralization capacity against an isolate of ancestral virus from the B.1 lineage containing the D614G substitution. Neutralization of this ancestral isolate had an $FRNT_{50}$ of 2038, substantially higher than BA.1 neutralization by the same plasma (Fig. 2a). Compared to ancestral virus, neutralization dropped 4.0-fold for BA.1, 12.9-fold for BA.4, and 10.3-fold for BA.5 (Fig. 2c).

Because the cohort contained participants vaccinated with the Johnson and Johnson Ad26.CoV2.S in addition to the Pfizer BNT162b2 vaccine (Table S1) and participants who differed in their HIV-1 status (14 were people living with HIV, of whom 13 were virologically suppressed with antiretroviral therapy[21]), we examined whether HIV status and vaccine type impacted our results by comparing the fold-drop in neutralization of BA.4 and BA.5 to BA.1 in the different subgroups. Within the vaccinated group, the fold-drop with BA.4 and BA.5 was very similar when comparing neutralization of sera from participants vaccinated with Ad26.CoV2.S versus BNT162b2 (Fig. S1a). Likewise, fold-drops in neutralization did not substantially change between vaccinated people living with HIV and HIV negative participants (Fig. S1b). In contrast, there was a trend with borderline significance that showed higher BA.4 and BA.5 escape in people living with HIV who were unvaccinated (Fig. S1c).

The L452R and F486V mutations in the spike receptor binding domain could potentially mediate escape from vaccine elicited neutralization independently of BA.1 infection elicited immunity. To test this, we measured BA.1, BA.4, and BA.5 neutralization relative to ancestral D614G virus in 18 vaccinated South African participants who did not have BA.1 breakthrough infection (Table S1). Because we have previously observed that Beta variant infection may broaden vaccine elicited neutralization capacity[23], we did not include participants previously infected with a variant and restricted this group to either individuals who were vaccinated only or vaccinated and infected with ancestral/D614G. Here neutralization declined from $FRNT_{50}$ = 4123 for ancestral/D614G to 208 for BA.1, 211 for BA.4 and 197 for BA.5 (Fig. 3a). BA.4 and BA.5 neutralization did not drop compared to BA.1 in this group (Fig. 3b). Fold-drops relative to ancestral virus were 19.8-fold for BA.1, 19.6-fold for BA.4 and 20.9-fold for BA.5 (Fig. 3c).

We observed that escape of BA.4 and BA.5 relative to BA.1 from neutralizing immunity was strongest in BA.1 infected unvaccinated individuals (Fig. 1e) and was moderated by vaccination in vaccinated people with BA.1 breakthrough infection (Fig. 2b). In contrast, BA.1, BA.4, and BA.5 showed similar (and extensive) escape in vaccinated people who did not have BA.1 infection elicited immunity (Fig. 3b).

BA.4 and BA.5 viruses showed very similar neutralization escape to each other, with minor differences which may be explained by experimental variation. This is expected since they share the same spike sequence, with the exception that our BA.4 isolate contained the N658S spike mutation found in a subset of BA.4 sequenced infections reported in GISAID (27% at the time of writing, see https://outbreak.info/compare-lineages?pango=BA.4&gene=S&threshold=10&nthresh=1&sub=false&dark=false) but not in BA.5. However, because we test neutralization against the live virus and not spike alone, we cannot rule out that the difference is real and occurs because of differences in the other genes (which may perhaps modulate neutralization by influencing spike surface expression or another parameter not directly related to spike sequence). In contrast to BA.4 and BA.5, we detected only minor escape of BA.2 from BA.1 elicited immunity in the same cohort of BA.1 infected individuals in a previous study[21].

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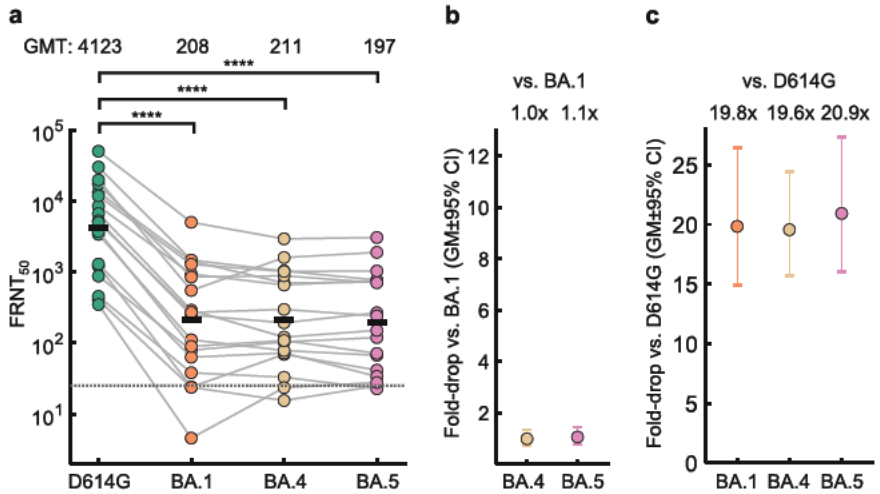

Fig. 3 | Escape of BA.4 and BA.5 from immunity elicited by vaccination combined in the absence of BA.1 infection. **a** Neutralization of ancestral/D614G, BA.1, BA.4 and BA.5 by vaccine elicited neutralizing immunity in $n = 18$ participants. Numbers are geometric mean titer (GMT) $FRNT_{50}$. Dashed line is most concentrated plasma tested. **b** Geometric mean (GM) of fold-drops in neutralization and their 95% confidence intervals for BA.4 and BA.5 relative to BA.1 calculated from (**a**). **c** GM of fold-drops in neutralization and their 95% confidence intervals for BA.1, BA.4 and BA.5 relative to ancestral/D614G virus calculated from (**a**). For all panels, green points are values for ancestral/D614G, orange points are BA.1, yellow points are BA.4, and pink points are BA.5. *p*-values were determined by a two-sided Wilcoxon rank sum test and represented as ****<0.0001. Exact *p*-values were 7.2×10^{-5} for BA.1, 3.2×10^{-5} for BA.4, and 2.4×10^{-5} for BA.5. Source data are provided as a Source Data file.

As we previously reported[21] and confirmed here, BA.1 elicits relatively weak neutralization in the absence of vaccination, consistent with reports showing that Omicron has reduced immunogenicity[24–26]. Even with BA.1 breakthrough infection, the $FRNT_{50}$ against ancestral virus was about half of that measured in a group composed mostly (Table S1) of people with ancestral infection and vaccination hybrid immunity (Fig. 3a). However, there are caveats to this comparison, including the order of infection and vaccination, with infection occurring first in the non-BA.1 infected group and the samples collected after vaccination.

Since our original release of the BA.4 and BA.5 neutralization results, other groups reported similar conclusions[27–31], with BA.4 and BA.5 escape from BA.1 and BA.2 elicited immunity being very similar to our measurements. These studies analyzed different cohorts from us and from each other, yet the results converged. Our cohort, which enrolls people who use the South African public health system, is generally distinguished from cohorts in other countries with active sero-surveillance of variants by the higher proportion of people who are unvaccinated, the higher proportion of people vaccinated with the Johnson and Johnson Ad26.CoV2.S vaccine, and the higher proportion of people who are living with HIV. In the vaccinated group we did not find evidence that either vaccine type or HIV status impacted the fold-drop in neutralization observed with BA.4 and BA.5 relative to BA.1. However, there was a trend to higher escape of BA.4 and BA.5 in unvaccinated individuals living with HIV. This is consistent with our previous results showing that the neutralization response elicited by a vaccine to a variant is similar between people living with HIV who are well suppressed with antiretroviral therapy and people who are HIV-negative, but that the response is attenuated by HIV in unvaccinated people[32].

A recent report showed that BA.4/BA.5 was more fusogenic in cell culture and more pathogenic relative to BA.2 in the hamster model[27]. However, despite this the BA.4/BA.5 infection wave in South Africa did not lead to a sharp increase in excess deaths associated with the other infection waves, although the association was also reduced in the BA.1 infection wave (Fig. 1a). This may indicate that, while SARS-CoV-2 pathogenicity continues to fluctuate and may evolve away from the attenuated pathogenicity observed in BA.1[33], the increased population immunity may keep disease severity relatively low[34]. Consistent with this, a recent analysis showed that neutralization capacity required to prevent severe disease is considerably lower than that required to prevent symptomatic infection[35]. In addition, there may be factors specific to the South African infection environment which reduce pathogenicity such as immunity from Beta infection combined with vaccination, which we found to broaden neutralization capacity against BA.4 and BA.5[23].

Limitations of this study include that we did not have enough participants with BA.2 infection or booster vaccination to test escape against this type of elicited immunity, which is much more common in some countries, for example those in Europe and North America. Our cohort is heterogeneous in terms of vaccination. Most participants are not vaccinated. Vaccinated participants are divided into two almost equal groups of Pfizer BNT162b2 and Johnson and Johnson Ad26.CoV2.S, though when we compared these vaccinated groups we observed that they were similar in terms of BA.4 and BA.5 escape. This may raise concerns that the heterogeneity in the relatively small vaccinated group may limit our ability to make more general conclusions about the degree of BA.4 and BA.5 immune escape in BA.1 infected vaccinated individuals.

Furthermore, the South African population differs from that of other countries where SARS-CoV-2 infection is intensively studied. South Africa has a lower fraction of vaccinated people, higher HIV prevalence, and people with previous immunity from an extensive Beta variant infection wave[22,23,36]. Every cohort is specific to the population it is drawn from, and it takes cohorts from multiple countries to get an accurate measure of immune escape of variants globally. The heterogeneity of individuals in our cohort reflects the heterogeneity in the South African population, and we chose not to limit our investigation to a specific subgroup. What may be specifically relevant in the population we study is that BA.4/BA.5, as well as BA.1, were first detected in South Africa and likely evolved in this region. Therefore, our study may indicate the selective forces at play in BA.4/BA.5 evolution. Given our observation that BA.4 and BA.5 have the strongest neutralization escape advantage in unvaccinated people, it may be important to determine whether the increasing vaccination coverage will reduce variant evolution.

Methods

Informed consent and ethical statement

Blood samples were obtained after written informed consent from adults with PCR-confirmed SARS-CoV-2 infection who were enrolled in a prospective cohort study at the Africa Health Research Institute approved by the Biomedical Research Ethics Committee at the University of KwaZulu Natal (reference BREC/00001275/2020). The Omicron/BA.1 and BA.4 was isolated from a residual swab sample with SARS-CoV-2 isolation from the sample approved by the University of the Witwatersrand Human Research Ethics Committee (HREC) (ref. M210752). The sample to isolate Omicron/BA.5 was collected after written informed consent as part of the COVID-19 transmission and natural history in KwaZulu-Natal, South Africa: Epidemiological Investigation to Guide Prevention and Clinical Care in the Centre for the AIDS Programme of Research in South Africa (CAPRISA) study and approved by the Biomedical Research Ethics Committee at the University of KwaZulu Natal (reference BREC/00001195/2020, BREC/00003106/2021). REDCap version 11.1.29 was used to collect participant data.

Whole-genome sequencing, genome assembly and phylogenetic analysis

RNA was extracted on an automated Chemagic 360 instrument, using the CMG-1049 kit (Perkin Elmer, Hamburg, Germany). The RNA was stored at 80 °C prior to use. Libraries for whole genome sequencing were prepared using either the Oxford Nanopore Midnight protocol with Rapid Barcoding or the Illumina COVIDseq Assay. For the Illumina COVIDseq assay, the libraries were prepared according to the manufacturer's protocol. Briefly, amplicons were fragmented, followed by indexing using the Nextera UD Indexes Set A. Sequencing libraries were pooled, normalized to 4 nM and denatured with 0.2 N sodium acetate. An 8 pM sample library was spiked with 1% PhiX (PhiX Control v3 adaptor-ligated library used as a control). We sequenced libraries on a 500-cycle v2 MiSeq Reagent Kit on the Illumina MiSeq instrument (Illumina). On the Illumina NextSeq 550 instrument, sequencing was performed using the Illumina COVIDSeq protocol (Illumina Inc, USA), an amplicon-based next-generation sequencing approach. The first strand synthesis was carried using random hexamers primers from Illumina and the synthesized cDNA underwent two separate multiplex PCR reactions. The pooled PCR amplified products were processed for tagmentation and adapter ligation using IDT for Illumina Nextera UD Indexes. Further enrichment and cleanup was performed as per protocols provided by the manufacturer (Illumina Inc). Pooled samples were quantified using Qubit 3.0 or 4.0 fluorometer (Invitrogen Inc.) using the Qubit dsDNA High Sensitivity assay according to manufacturer's instructions. The fragment sizes were analyzed using TapeStation 4200 (Invitrogen). The pooled libraries were further normalized to 4 nM concentration and 25 µL of each normalized pool containing unique index adapter sets were combined in a new tube. The final library pool was denatured and neutralized with 0.2 N sodium hydroxide and 200 mM Tris-HCL (pH7), respectively. 1.5 pM sample library was spiked with 2% PhiX. Libraries were loaded onto a 300-cycle NextSeq 500/550 HighOutput Kit v2 and run on the Illumina NextSeq 550 instrument (Illumina, San Diego, CA, USA). For Oxford Nanopore sequencing, the Midnight primer kit was used as described by Freed and Silander55. cDNA synthesis was performed on the extracted RNA using LunaScript RT mastermix (New England BioLabs) followed by gene-specific multiplex PCR using the Midnight Primer pools which produce 1200 bp amplicons which overlap to cover the 30-kb SARS-CoV-2 genome. Amplicons from each pool were pooled and used neat for barcoding with the Oxford Nanopore Rapid Barcoding kit as per the manufacturer's protocol. Barcoded samples were pooled and bead-purified. After the bead clean-up, the library was loaded on a prepared R9.4.1 flow-cell. A GridION X5 or MinION sequencing run was initiated using MinKNOW software with the base-call setting switched off. We assembled paired-end and nanopore.fastq reads using Genome Detective v2.40 (https://www.genomedetective.com) which was updated for the accurate assembly and variant calling of tiled primer amplicon Illumina or Oxford Nanopore reads, and the Coronavirus Typing Tool56. For Illumina assembly, GATK HaploTypeCaller --min-pruning 0 argument was added to increase mutation calling sensitivity near sequencing gaps. For Nanopore, low coverage regions with poor alignment quality (<85% variant homogeneity) near sequencing/amplicon ends were masked to be robust against primer drop-out experienced in the Spike gene, and the sensitivity for detecting short inserts using a region-local global alignment of reads, was increased. In addition, we also used the wf artic (ARTIC SARS-CoV-2 pipeline, v0.3.18) as built using the nextflow workflow framework57. In some instances, mutations were confirmed visually with.bam files using Geneious software V2020.1.2 (Biomatters). The reference genome used throughout the assembly process was NC 045512.2 (numbering equivalent to MN908947.3.

Cells

Vero E6 cells (originally ATCC CRL-1586, obtained from Cellonex in South Africa) were propagated in complete growth medium consisting of Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (Hyclone) containing 10 mM of hydroxyethylpiperazine ethanesulfonic acid (HEPES), 1 mM sodium pyruvate, 2 mM L-glutamine and 0.1 mM nonessential amino acids (Sigma Aldrich). Vero E6 cells were passaged every 3 4 days. The H1299-E3 cell line (H1299 originally from ATCC as CRL-5803) was propagated in growth medium consisting of complete Roswell Park Memorial Institute (RPMI) 1640 medium with 10% fetal bovine serum containing 10 mM of HEPES, 1 mM sodium pyruvate, 2 mM L-glutamine and 0.1 mM nonessential amino acids. Cells were passaged every second day. The H1299-E3 (H1299-ACE2, clone E3) cell line was derived from H1299 as described in our previous work[1,22]. Briefly, vesicular stomatitis virus G glycoprotein (VSVG) pseudotyped lentivirus containing hACE2 was used to infect H1299 cells. ACE-2 transduced H1299 cells were subcloned at the single cell density in 96-well plates (Eppendorf) in conditioned media derived from confluent cells. After 3 weeks, wells were detached using a 0.25% trypsin-EDTA solution (Gibco) and plated in two replicate plates, where the first plate was used to determine infectivity and the second was stock. The first plate was screened for the fraction of mCherry positive cells per cell clone upon infection with a SARS-CoV-2 mCherry expressing spike pseudotyped lentiviral vector. Screening was performed using a Metamorph-controlled (Molecular Devices, Sunnyvale, CA) Nikon TiE motorized microscope (Nikon Corporation, Tokyo, Japan) with a 20x, 0.75 NA phase objective, 561 nm laser line, and 607 nm emission filter (Semrock, Rochester, NY). Images were captured using an 888 EMCCD camera (Andor). The clone with the highest fraction of mCherry expression was expanded from the stock plate and denoted H1299-E3. Cell lines have not been authenticated. The cell lines have been tested for mycoplasma contamination and are mycoplasma negative.

Virus expansion

All work with live virus was performed in Biosafety Level 3 containment using protocols for SARS-CoV-2 approved by the Africa Health Research Institute Biosafety Committee. ACE2-expressing H1299-E3 cells were seeded at 4.5×10^5 cells in a 6 well plate well and incubated for 18 20 h. After one Dulbecco's phosphate-buffered saline (DPBS) wash, the sub-confluent cell monolayer was inoculated with 500 µL universal transport medium diluted 1:1 with growth medium filtered through a 0.45-µm filter. Cells were incubated for 1 h. Wells were then filled with 3 mL complete growth medium. After 4 days of infection (completion of passage 1 (P1)), cells were trypsinized, centrifuged at $300 \times g$ for 3 min and resuspended in 4 mL growth medium. Then all infected cells were added to Vero E6 cells that had been seeded at

1.5×10^5 cells per mL, 20 mL total, 18 20 h earlier in a T75 flask for cell-to-cell infection. The coculture of ACE2-expressing H1299-E3 and Vero E6 cells was incubated for 1 h and the flask was filled with 20 mL of complete growth medium and incubated for 4 days. The viral supernatant from this culture (passage 2 (P2) stock) was used for experiments.

Live virus neutralization assay

H1299-E3 cells were plated in a 96-well plate (Corning) at 30,000 cells per well 1 day pre-infection. Plasma was separated from EDTA-anticoagulated blood by centrifugation at $500 \times g$ for 10 min and stored at 80 °C. Aliquots of plasma samples were heat-inactivated at 56 °C for 30 min and clarified by centrifugation at $10{,}000 \times g$ for 5 min. Virus stocks were used at approximately 50 100 focus-forming units per microwell and added to diluted plasma. Antibody virus mixtures were incubated for 1 h at 37 °C, 5% CO_2. Cells were infected with 100 µL of the virus antibody mixtures for 1 h, then 100 µL of a 1X RPMI 1640 (Sigma Aldrich, R6504), 1.5% carboxymethylcellulose (Sigma Aldrich, C4888) overlay was added without removing the inoculum. Cells were fixed 18 h post-infection using 4% PFA (Sigma-Aldrich) for 20 min. Foci were stained with a primary rabbit anti-spike monoclonal antibody (BS-R2B12, GenScript A02058) at 0.5 µg/mL in a permeabilization buffer containing 0.1% saponin (Sigma-Aldrich), 0.1% bovine serum albumin (BSA, Sigma Aldrich) and 0.05% Tween-20 (Sigma Aldrich) in phosphate-buffered saline (PBS) overnight at 4 °C, then washed with wash buffer containing 0.05% Tween-20 in PBS. Secondary goat anti-rabbit horseradish peroxidase (HRP) conjugated antibody (Abcam ab205718) was added at 1 µg/mL and incubated for 2 h at room temperature with shaking. TrueBlue peroxidase substrate (SeraCare 5510-0030) was then added at 50 µL per well and incubated for 20 min at room temperature. Plates were imaged in an ImmunoSpot Ultra-V S6-02-6140 Analyzer ELISPOT instrument with BioSpot Professional built-in image analysis (C.T.L).

Statistics and fitting

All statistics and fitting were performed using custom code in MATLAB v.2019b. Neutralization data were fit to:

$$Tx = 1/1 + (D/ID_{50}). \quad (1)$$

Here Tx is the number of foci normalized to the number of foci in the absence of plasma on the same plate at dilution D and ID_{50} is the plasma dilution giving 50% neutralization. $FRNT_{50} = 1/ID_{50}$. Values of $FRNT_{50} < 1$ are set to 1 (undiluted), the lowest measurable value. We note that the most concentrated plasma dilution was 1:25 and therefore $FRNT_{50} < 25$ were extrapolated. To calculate confidence intervals, $FRNT_{50}$ or fold-change in $FRNT_{50}$ per participant was log transformed and arithmetic mean plus 2 std and arithmetic mean minus 2 std were calculated for the log transformed values. These were exponentiated to obtain the upper and lower 95% confidence intervals on the geometric mean $FRNT_{50}$ or the fold-change in $FRNT_{50}$ geometric means.

Data availability

Sequences of outgrown Omicron sub-lineage isolates have been deposited to GenBank with accession codes as follows: Ancestral virus, B.1 lineage, with the D614G substitution, OP090658. Omicron/BA.1, OP090659. Omicron/BA.4, OP093374. Omicron/BA.5, OP093373 and have also been deposited in GISAID with accession codes and hyperlinks as follows: Ancestral virus, B.1 lineage, with the D614G substitution, EPI ISL 602626.1 [https://www.epicov.org/epi3/frontend#357674]. Omicron/BA.1, EPI ISL 7886688 [https://www.epicov.org/epi3/frontend#6274a9]. Omicron/BA.4, EPI ISL 12268495.2 [https://www.epicov.org/epi3/frontend#434eae]. Omicron/BA.5, EPI ISL 12268493.2 [https://www.epicov.org/epi3/frontend#49d7ec]. Source data are provided with this paper.

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Author contributions

A.S. and K.K. conceived the study and designed the study and experiments. A.vG., D.M., N.W., Q.A.K., S.S.A.K., G.L., A.Si, and N.S. identified and provided virus samples. M.-Y.S.M., F.K., B.I.G., M.B., K.K., N.M., N.Ma, M.M., Y.M., N.N., Z.J., K.R., and Y.G. set up and managed the cohort and cohort data. K.K., Z.J., K.R., S.C., Y.G., H.T., J.E.S., J.G., Y.N., S.P., performed experiments and sequence analysis with input from A.S., T.dO., R.J.L. A.S., K.K., and M.B. interpreted data with input from M-Y.S.M., S.S.A.K., W.H., T.dO., R.J.L. A.S. and K.K. prepared the manuscript with input from all authors.

Competing interests

The authors declare no competing interests.

Additional information

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CHAPTER 5: GENERAL DISCUSSION

The South African population differs from other countries where SARS-CoV-2 infection is intensively studied. South Africa has a higher HIV prevalence rate (Kharsany et al., 2018, Govere-Hwenje et al., 2022), a lower fraction of vaccinated people, and people with previous immunity from an extensive Beta infection wave (Khan et al., 2021). It is also the geographical area where Beta and Omicron was first detected and isolated (Cele et al., 2021b).

Concerns about variants include whether a variant is more infectious or severe than previous VoCs and whether it can circumvent vaccine protection. Four key concerns stemming from the emergence of new variants are their effects on viral transmissibility, disease severity, reinfection rates due to escape, and vaccine effectiveness or escape from vaccine-induced immunity (Abdool Karim and de Oliveira, 2021). This suggests that, despite the number of people who have already been infected with SARS-CoV-2 globally and are presumed to have accumulated some level of immunity, new variants can pose a substantial re-infection risk (Wibmer et al., 2021).

Omicron and Omicron sub-lineages, seem to be the most antigenically divergent in comparison to other variants, and outcompeted other variants in the context of pre-existing immunity, infection, vaccination, or a combination (Chalkias et al., 2022, Pulliam et al., 2022). Vaccination strategies that can induce more potent, durable, and broader immune responses are important to enhance protection.

The unifying theme which emerges from this thesis is the ability of hybrid immunity which combines infection and vaccination to compensate for antibody neutralization escape mutations which arise in emerging variants, as well as the reduced effectiveness of the neutralizing immune response due to HIV and particularly poorly controlled HIV infection. This was demonstrated in this work by: 1) lower neutralizing capacity against the Delta variant in PLWH with immunity from previous infection only but not in PLWH with hybrid immunity; 2) the substantially higher cross-neutralization of Delta, and Omicron BA.2, BA.4, and BA.5 subvariants in individuals with Omicron BA.1 infection/vaccination immunity relative to those with immunity elicited with BA.1 infection alone. Therefore, the declining vaccination rate in South Africa and throughout Sub-Saharan Africa is particularly concerning. This may mean that future immunity will be elicited primarily from infection alone and may not give sufficient cross-protection from emerging variants or to individuals with a reduced capacity to elicit a potent neutralizing antibody response.

CHAPTER 6: REFERENCES

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