Limited cross-variant immune response from SARS-CoV-2 Omicron BA.2 in naïve but not previously infected outpatients

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The diagram illustrates the differences in antibody titers and immune transcriptome between individuals with no prior infection and those with prior infection. The Omicron BA.1 and BA.2 variants are compared.

**Omicron**
- BA.1: No prior infection
- BA.2: No prior infection

**BA.1**
- Prior infection

**BA.2**
- Prior infection

**Antibody titers**
- BA.1: No prior infection
- BA.2: Prior infection

**Log2 FC (Expression Change)**
- BA.1 > BA.2
- No prior infection > Prior infection

**Immune transcriptome**
- IG genes
Limited cross-variant immune response from SARS-CoV-2 Omicron BA.2 in naïve but not previously infected outpatients

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SUMMARY

Omicron is currently the dominant SARS-CoV-2 variant and several sublineages have emerged. Questions remain about the impact of previous SARS-CoV-2 exposure on cross-variant immune responses elicited by the SARS-CoV-2 Omicron sublineage BA.2 compared to BA.1. Here we show that without previous history of COVID-19, BA.2 infection induces a reduced immune response against all variants of concern (VOC) compared to BA.1 infection. The absence of ACE2 binding in sera of previously naïve BA.1 and BA.2 patients indicates a lack of meaningful neutralization. In contrast, anti-spike antibody levels and neutralizing activity greatly increased in the BA.1 and BA.2 patients with a previous history of COVID-19. Transcriptome analyses of peripheral immune cells showed significant differences in immune response and specific antibody generation between BA.1 and BA.2 patients as well as significant differences in expression of specific immune genes. In summary, prior infection status significantly impacts the innate and adaptive immune response against VOC following BA.2 infection.

INTRODUCTION

a Senior authors
The highly transmissible SARS-CoV-2 Omicron (B.1.1.529) variant has replaced previous variants and is less susceptible to neutralizing antibodies elicited by vaccination or infection (Cele et al., 2022; Edara et al., 2022; Schmidt et al., 2022). Currently, BA.2 is the dominant Omicron sublineage and has replaced BA.1 (Viana et al., 2022). While BA.1 and BA.2 share 32 mutations, they differ in 28 and demonstrate different antibody neutralization profiles (Arora et al. 2022a). Omicron spike mutations negatively impact neutralizing activity in sera from previously vaccinated and previously infected persons as well as limited efficacy against monoclonal antibody treatment (Dejnirattisai et al., 2022; Hoffmann et al., 2022; Mannar et al., 2022; VanBlargan et al., 2022). Of note, vaccinated individuals with BA.1 infection develop measurable neutralizing antibody titers against BA.1 and BA.2 (Yu et al., 2022). Prior vaccination also broadens the immunological response against variants of concern (VOC) following Omicron infection whereas unvaccinated individuals show an antibody response more limited to the infecting strain (Lee et al., 2022b; Suryawanshi et al., 2022).

The ability of BA.2 infection to induce neutralizing antibodies in unvaccinated individuals, either without or with previous SARS-CoV-2 infection, is pending definition. Here we conducted a comparative investigation of the innate and humoral immune response elicited in 74 Omicron outpatients infected with BA.1 (Lee et al., 2022b) or BA.2 that were either naïve prior to Omicron infection or had prior exposure to other SARS-CoV-2 variants. We measured antibody titers and ACE2 binding inhibition as a proxy to neutralization. In addition, we investigated the immune transcriptome and the repertoire of antibody germline alleles in these cohorts.

RESULTS
Antibody response after Omicron subvariant infection
We measured antibody titers and neutralizing antibody responses in 74 outpatients infected with either Omicron BA.1 or BA.2 sublineages. Demographic and clinical information are provided in Table 1. While none of the patients had been vaccinated, 24 had been infected by an earlier variant. More than 20% of the BA.1 infected, but none of the BA.2 infected patients, had moderate or severe disease. Humoral immune response was measured between 7 and 21 days (median: days 13-14) post documented Omicron
In previously uninfected and unvaccinated individuals, anti-Omicron BA.1 spike IgG titers were approximately 40-fold lower following BA.2 infection compared to BA.1 infection (Figure 1A; Table S1). IgG titers against the ancestral spike were almost 70-fold lower following BA.2 as compared to BA.1 infection. Anti-spike IgG antibody titers in individuals previously infected with earlier SARS-CoV-2 variants were at least 5-fold higher with no difference in titers between those infected with the BA.1 versus BA.2 variants (Figure 1A; Table S1). This highlights that previous infection with variants has a particular strong impact on the humoral immune response in BA.2 infected patients.

For reference, titers from individuals infected with the Omicron Beta variant (Knabl et al., 2022) are shown. These approximate those obtained in individuals previously infected with an earlier variant who then experienced BA.1 or BA.2 infection. The differential response in antibody generation following BA.1 and BA.2 infection was consistent across all SARS-CoV-2 variants tested (Figure 1B).

We next measured neutralization capacity using the angiotensin-converting enzyme 2 (ACE2) binding inhibition assay (Figure 1C). Neutralizing activities against the ancestral strain and Omicron BA.1 were similarly low in antigen naïve individuals infected with either BA.1 or BA.2. Like the overall antibody titers, neutralizing activity was higher in those previously infected. Activity from BA.1 and BA.2 infected individuals with previous SARS-CoV-2 infection approximated that found for individuals infected with the Omicron Beta variant and was similar across all SARS-CoV-2 variants tested against (Figure 1D).

**Transcriptome response**

Next, we investigated the impact of BA.1 and BA.2 infection on the immune transcriptome in Omicron patients with or without a previous infection history (Figure 2; Table S2: Significantly differential genes between BA.1 and BA.2 infected cohorts with no prior infection; Table S3: Significantly differential genes between no prior and prior infection in BA.2 infected cohorts). Buffy coats were isolated within the first three days after validated Omicron infection and bulk RNA-seq was conducted with an average sequencing depth of 200 million reads per sample. First, we directly compared the transcriptomes of the BA.1 and BA.2 infected Omicron cohorts without prior infection. In a direct comparison of
the two cohorts, expression of 38 and 15 genes was induced significantly in patients infected with BA.1 and BA.2, respectively (Figure 2A; Table S2: Significantly differential genes between BA.1 and BA.2 infected cohorts with no prior infection). While genes activated upon BA.1 infection are enriched in neutrophil degranulation and innate immune system, the ones in activated upon BA.2 infection are categorized in complement pathway (Figure 2B; Table S2: Significantly differential genes between BA.1 and BA.2 infected cohorts with no prior infection; Figure S1). Genes induced at significantly higher levels in BA.1 patients, such as C4BPA, initially identified by RNA-seq analyses (Figure 2C) and validated by qRT-PCR (Figure S1A), have also been reported to be regulated in COVID-19 patients (Gutmann et al., 2021; Jabeen et al., 2022). Of note, the complement genes C1QA and C1QB that show elevated expression in nonclassical monocytes from COVID-19 patients (Stephenson et al., 2021) are preferentially expressed in BA.2 patients as initially identified by RNA-seq analyses (Figure 2C) and validated by qRT-PCR (Figure S1B). We then compared the immune transcriptomes from BA.2 patients with and without prior infection and identified 342 and 145 significantly induced genes, respectively (Figure 2D; Table S3: Significantly differential genes between no prior and prior infection in BA.2 infected cohorts). GSEA analyses linked the induced genes in the ‘no prior infection’ cohort to innate immune responses, including interferon and inflammatory pathways (Figure 2E). Genes activated in the ‘no prior infection’ cohort such as CCL2 and IL6, initially identified by RNA-seq (Figure 2F) and validated by qRT-PCR (Figure S1C), are part of the interferon response in severe COVID-19 (Cao, 2021; Munnur et al., 2021). Similar to what has been previously reported for a transcriptome analysis of peripheral blood mononuclear cells of SARS-CoV-2 infected patients, in general, there was good agreement between RNA-seq (Figure 2) and qRT-PCR results for genes differentially expressed between different cohorts (Figure S1) (Zhang et al., 2021b).

**Antibody germline repertoire**

The higher level of anti-spike antibodies and neutralization activity in BA.1 patients of the ‘no prior infection’ group compared to BA.2 infection, led us to dig deeper and interrogate the germline antibody gene usage. Specifically, we determined the range of immunoglobulin heavy chain (IGHV) gene usage elicited by BA.1 and BA.2 sublineage
infection in the ‘no previous infection’ cohort (Figure 3). RNA-seq was conducted 13-16 days after the positive PCR test. The number of total immunoglobulin genes (heavy chain variable (IGHV), light chain variable (IGKV and IGLV) and T cell receptor alpha and beta variable (TRAV and TRBV)) revealed the use of a broad range of germlines in all cohorts without statistically significance (Figure 3A). However, we observed increased frequency of transcription of eight VH genes, including IGHV3-11, IGHV3-66, IGHV1-2 and IGHV4-61, in the BA.1 infection group compared to the BA.2 cohort (Figure 3B). These particular heavy chain variants have been identified in SARS-CoV-2 infected patients and COVID-19 vaccinated individuals that developed strongly neutralizing antibodies (Andreano et al., 2021; Andreano and Rappuoli, 2021; Collier et al., 2021; He et al., 2021; Lee et al., 2022a; Zhang et al., 2021a). Expression of some of these genes was also activated in octogenarians which had received a single dose of the BNT162b mRNA vaccine 15 months after recovery from COVID-19 (Lee et al., 2022a).

DISCUSSION

While the Omicron BA.1 and BA.2 sublineages share mutations they also carry unique mutations, 28 in BA.2, which can explain their differential response to vaccine-induced and therapeutic antibodies (Ai et al., 2022; Arora et al., 2022a; Arora et al., 2022b). Previous studies have also demonstrated that BA.1 infection of naïve persons yields a very limited antibody response to SARS-CoV-2 VOCs other than Omicron itself (Lee et al., 2022b; Rössler et al., 2022). However, prior vaccination or prior infection with older SARS-CoV-2 variants augment the immune response upon BA.1 breakthrough infection (Lee et al., 2022b; Rössler et al., 2022). Our study now reveals that in antigen naïve individuals the immunologic response following BA.2 infection is even lower than that elicited by BA.1 infection as indicated by significantly lower antibody titers against all VOCs.

While other studies have focused on the humoral response including neutralizing activity induced by Omicron infection (Suryawanshi et al., 2022), we have extended the investigations and explored the presence of germline immunoglobulin variants induced by BA.1 and BA.2 infection. RBD-targeting antibodies can block SARS-CoV-2 binding to ACE2 and neutralizing immunoglobulin G heavy-chain variable (IGHV) gene usage has
been reported (Yuan et al., 2020). Our study identified several IGHV germline alleles in Omicron patients that have been identified in neutralizing antibodies in COVID-19 patients (Yan et al., 2021; Yuan et al., 2020).

In conclusion, our study demonstrates that Omicron BA.2 infection of unvaccinated individuals without prior COVID-19 does not elicit effective antibodies against BA.1 and non-Omicron variants. In previously infected individuals, however, BA.2 infection successfully induces immunity against other variants.

**Limitations of the study**

There are several limitations to the current study. First, the study was conducted on volunteers from a specific geographical area, Tyrol (Austria). Secondly, limitation is the confinement of the study to a timeframe of three weeks following Omicron infection. Third, the number of BA.2 patients with prior infection was very limited. Additional individuals for this cohort could not be recruited due to replacement of BA.2 infection by BA.4 and BA.5 in Tyrol. Similar pandemic-related cohort size limitations have been noted for other transcriptome-based COVID studies (Sureshchandra et al., 2021; Zhang et al., 2021b).

Lastly, some of the data collected on breakthrough infections was reliant on retrospective chart review and not collected as part of a prospective study.

**ACKNOWLEDGMENTS**

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Our gratitude goes to the participants who contributed to this study to advance our understanding of SARS-CoV-2 Omicron infection. We thank Yuhai Dai from the NIDDK clinical core for helping antibody assay. This work was utilized the computational resources of the NIH HPC Biowulf cluster (http://hpc.nih.gov). RNA-sequencing and single cell RNA-sequencing was conducted in the NIH Intramural Sequencing Center, NISC (https://www.nisc.nih.gov/contact.htm)

**AUTHOR CONTRIBUTIONS**
H.K.L., L.K. and L.H. designed the study. L.K recruited patients and collected material. H.K.L. analyzed RNA-seq data. M.W. conducted antibody assays. H.K.L., L.K., P.A.F. and L.H. analyzed data. L.H. supervised project. H.K.L., L.K., P.A.F. and L.H. wrote the paper. All authors read and approved the manuscript.

DECLARATION OF INTERESTS
The authors declare not competing interests.

INCLUSION AND DIVERSITY
We worked to ensure gender balance in the recruitment of human subjects. We worked to ensure ethnic or other types of diversity in the recruitment of human subjects. We worked to ensure that the study questionnaires were prepared in an inclusive way. One or more of the authors of this paper self-identifies as an underrepresented ethnic minority in their field of research or within their geographical location. One or more of the authors of this paper self-identifies as a gender minority in their field of research. One or more of the authors of this paper self-identifies as living with a disability. While citing references scientifically relevant for this work, we also actively worked to promote gender balance in our reference list. We avoided “helicopter science” practices by including the participating local contributors from the region where we conducted the research as authors on the paper.

STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Biological Samples  |        |            |
| Human serum         | Tyrol, Austria | N/A |
| Human immune cells  | Tyrol, Austria | N/A |
| Critical Commercial Assays |        |            |
| Maxwell RSC simply RNA Blood Kit | Promega | Cat# ASB1380 |
| V-PLEX SARS-CoV-2 Panel 23 (IgG) Kit | Meso Scale Discovery | Cat# K15567U |
| V-PLEX SARS-CoV-2 Panel 23 (ACE2) Kit | Meso Scale Discovery | Cat# K15570U |
| TruSeq Stranded mRNA Library Prep Kit | Illumina | Cat# RS-20020595 |
| SuperScript III First-Strand Synthesis SuperMix | Invitrogen | Cat#18080-400 |
| Item                                                                 | Supplier              | Catalog Number |
|----------------------------------------------------------------------|-----------------------|----------------|
| SsoAdvanced Universal SYBR Green Supermix                            | Bio-Rad               | Cat#1725271    |
| Human C4BPA primer set                                               | Realtimeprimers.com   | Cat# VHPS-1289 |
| Human ITLN1 primer set                                               | Realtimeprimers.com   | Cat# VHPS-4672 |
| Human MMP8 primer set                                                | Realtimeprimers.com   | Cat# VHPS-5768 |
| Human OLFM4 (GW112) primer set                                       | Realtimeprimers.com   | Cat# VHPS-3935 |
| Human OLR1 primer set                                                | Realtimeprimers.com   | Cat# VHPS-6460 |
| Human C1QA primer set                                                | Realtimeprimers.com   | Cat# VHPS-1148 |
| Human C1QB primer set                                                | Realtimeprimers.com   | Cat# VHPS-1149 |
| Human CCL2 primer set                                                | Realtimeprimers.com   | Cat# VHPS-1614 |
| Human CD83 primer set                                                | Realtimeprimers.com   | Cat# VHPS-1701 |
| Human IL6 primer set                                                 | Realtimeprimers.com   | Cat# VHPS-4559 |
| Human FOSB primer set                                                | Realtimeprimers.com   | Cat# VHPS-3373 |
| Human AXL primer set                                                 | Realtimeprimers.com   | Cat# VHPS-698  |
| Human Housekeeping Gene Set                                          | Realtimeprimers.com   | Cat# HHK-1     |

**Deposited Data**

- **Raw and analyzed data**: This paper, GEO: GSE205244
- **Omicron patient (Days 2-3)**: Lee et al., 2022b, GEO: GSE201530
- **Human reference genome UCSC, hg19**: UCSC Genome Browser, http://hgdownload.soe.ucsc.edu/downloads.html#mouse

**Software and Algorithms**

- **MSD DISCOVERY WORKBENCH analysis software**: https://www.mesoscale.com/en/products_and_services/software
- **Trimmomatic (version 0.36)**: Bolger et al., 2014, http://www.usadellab.org/cms/?page=trimmomatic
- **STAR (2.5.4a)**: Dobin et al., 2013, https://anaconda.org/bioconda/star/files?version=2.5.4a
- **HTSeq**: Anders et al., 2015, https://htseq.readthedocs.io/en/master/
- **R (3.6.3)**: https://www.R-project.org/
- **Bioconductor**: Huber et al., 2015, https://www.bioconductor.org/
- **DESeq2**: Love et al., 2014, https://bioconductor.org/packages/release/bioc/html/DESeq2.html
- **RUSeq**: Risso et al., 2014, https://bioconductor.org/packages/release/bioc/html/RIUSeq.html
- **dplyr**: https://CRAN.R-project.org/package=dplyr
- **ggplot2**: Wickham, 2009, https://ggplot2.tidyverse.org/
**GSEA**

| Tool                  | Website/Reference                          |
|-----------------------|--------------------------------------------|
| GSEA                  | https://www.gsea-msigdb.org/gsea/msigdb    |
| MiXCR                 | Bolotin et al., 2015                       |
| PRISM GraphPad (9.0.0)|                                           |

**RESOURCE AVAILABILITY**

**Lead Contact**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact Lothar Hennighausen (lotharh@nih.gov).

**Materials Availability**

This study did not generate new unique reagents.

**Data and Code Availability**

- RNA-seq data generated from this study were deposited under the accession GSE205244 in the Gene Expression Omnibus (GEO).
- RNA-seq data of Omicron patients were obtained under GSE201530.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

**Study population, study design and recruitment**

Data from a total of 74 patients that were unvaccinated against COVID19 and infected with either Omicron BA.1 or BA.2 were analyzed. 13 BA.2 patients and 10 new BA.1 patients were recruited for the study under informed consent with no history of prior vaccination and prior infection with other SARS-CoV-2 variants and 24 unvaccinated patients with prior infections (Table 1). Recruitment and blood sample collection took
place between December 2021 and March 2022. All samples identified from individuals with BA.1 and BA.2 infection within the community within this timeframe who provided consent were included. A resource constraint was that the specific sub-sample sizes were determined after recruitment. The smallest sub-sample was BA.2 infected individuals with history of prior infection (n=3) as compared to BA.2 infected individuals without prior infection (n=10), BA.1 infected individuals with history of prior infection (n=21) and BA.1 infected individuals without history of prior infection (n=40). Day numbers for samples refers to the number of days following initial positive SARS-CoV-2 RT-PCR test. An additional 53 BA.1 patients from our previous study (Lee et al., 2022b) were included.

Patient recruitment was performed by a medic who assessed clinical status including performance of an oxygen saturation (SpO2) test. The clinical spectrum of the patients’ SARS-CoV-2 symptoms were classified based on the National Institutes of Health (NIH) treatment guidelines (https://files.covid19treatmentguidelines.nih.gov/guidelines/section/section_43.pdf). This study was approved (EK Nr: 1064/2021) by the Institutional Review Board (IRB) of the Office of Research Oversight/Regulatory Affairs, Medical University of Innsbruck, Austria, which is responsible for all human research studies conducted in the State of Tyrol (Austria). The investigators do not need to have an affiliation with the University of Innsbruck. Written informed consent was obtained from all subjects. Participant information was coded and anonymized. This study was determined to impose minimal risk on participants. All methods were carried out in accordance with relevant guidelines and regulations. All research has been performed in accordance with the Declaration of Helsinki (https://www.wma.net/policies-post/wma-declaration-of-helsinkiethical-principles-for-medical-research-involving-human-subjects/). In addition, we followed the ‘Sex and Gender Equity in Research – SAGER – guidelines’ and included sex and gender considerations where relevant.

METHOD DETAILS

Antibody assay
Whole blood was collected by medical personnel after subjects had tested positive for SARS-CoV-2. Antibody containing sera were obtained by centrifuging EDTA blood samples for 10 min at 4,000g. End-point binding IgG antibody titers to various SARS-CoV-2–derived antigens were measured using the Meso Scale Discovery (MSD) platform. SARS-CoV-2 spike, nucleocapsid, Alpha, Beta, Gamma, Delta, and Omicron spike subdomains were assayed using the V-plex multispot COVID-19 serology kits (Panel 23 (IgG) Kit, K15567U). Plates were coated with the specific antigen on spots in the 96 well plate and the bound antibodies in the samples (1:50000 dilution) were then detected by anti-human IgG antibodies conjugated with the MSD SULPHO-TAG which is then read on the MSD instrument which measures the light emitted from the tag.

ACE2 binding inhibition (Neutralization) ELISA
The V-PLEX COVID-19 ACE2 Neutralization kit (Meso Scale Discovery, Panel 23 (ACE2) Kit, K15570U) was used to quantitatively measure antibodies that block the binding of ACE2 to its cognate ligands (SARS-CoV-2 and variant spike subdomains). Plates were coated with the specific antigen on spots in the 96 well plate and the bound antibodies in the samples (1:10 dilution) were then detected by Human ACE2 protein conjugated with the MSD SULPHO-TAG which is then read on the MSD instrument which measures the light emitted from the tag.

Extraction of the buffy coat and purification of RNA
Whole blood was collected, and total RNA was extracted from the buffy coat and purified using the Maxwell RSC simply RNA Blood Kit (Promega) according to the manufacturer’s instructions. The concentration and quality of RNA were assessed by an Agilent Bioanalyzer 2100 (Agilent Technologies, CA).

mRNA sequencing (mRNA-seq) and data analysis
The Poly-A containing mRNA was purified by poly-T oligo hybridization from 1 mg of total RNA and cDNA was synthesized using SuperScript III (Invitrogen, MA). Libraries for sequencing were prepared according to the manufacturer’s instructions with TruSeq Stranded mRNA Library Prep Kit (Illumina, CA, RS-20020595) and paired-end
sequencing was done with a NovaSeq 6000 instrument (Illumina) yielding 200-350 million reads per sample.

The raw data were subjected to QC analyses using the FastQC tool (version 0.11.9) (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). mRNA-seq read quality control was done using Trimmomatic (Bolger et al., 2014) (version 0.36) and STAR RNA-seq (Dobin et al., 2013) (version STAR 2.5.4a) using 150 bp paired-end mode was used to align the reads (hg19). HTSeq (Anders et al., 2015) (version 0.9.1) was to retrieve the raw counts and subsequently, Bioconductor package DESeq2 (Love et al., 2014) in R (https://www.R-project.org/) was used to normalize the counts across samples (Zhao et al., 2021) and perform differential expression gene analysis. Additionally, the RUVSeq (Risso et al., 2014) package was applied to remove confounding factors. The data were pre-filtered keeping only genes with at least ten reads in total. The visualization was done using dplyr (https://CRAN.R-project.org/package=dplyr) and ggplot2 (Wickham, 2009). The genes with log2 fold change >1 or < -1 and adjusted p-value (pAdj) <0.05 corrected for multiple testing using the Benjamini-Hochberg method were considered significant and then conducted gene enrichment analysis (GSEA, https://www.gSEA-msigdb.org/gsea/msigdb). DESeq2 is an established recommended program for analysis of RNA-seq data for ≥ 3 replicates per condition (Schurch et al., 2016).

For T- or B-cell receptor repertoire sequencing analysis, trimmed fastq files from bulk RNA-seq were aligned against human V, D and J gene sequences using the default settings with MiXCR (Bolotin et al., 2017; Bolotin et al., 2015).

**Quantitative real-time PCR (qRT–PCR)**

Total RNA (1 μg) was reverse transcribed for 50 min at 50°C using 50 μM oligo dT and 2 μl of SuperScript III First-Strand Synthesis SuperMix (Invitrogen) in a 20 μl reaction. Quantitative real-time PCR (qRT-PCR) was performed using primer sets (C4BPA, VHPS-1289; ITLN1, VHPS-4672; MMP8, VHPS-5768; OLFM4 (GW112), VHPS-3935; OLR1, VHPS-6460; C1QA, VHPS-1148; C1QB, VHPS-1149; CCL2, VHPS-1614; CD83, VHPS-1701; IL6, VHPS-4559; FOSB, VHPS-3373; AXL, VHPS-698; Human Housekeeping Gene Set, HHK-1, Realtimeprimers.com) and SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) on the CFX384 Real-Time PCR Detection System (Bio-Rad).
according to the manufacturer’s instructions. PCR conditions were 95°C for 10s and 58°C for 45s for 50 cycles. All reactions were done in triplicate and normalized to the housekeeping gene GAPDH. Relative differences in PCR results were calculated using the comparative cycle threshold ($C_T$) method.

**Quantification and statistical analysis**

Differential expression gene (DEG) identification used Bioconductor package DESeq2 in R. P-values were calculated using a paired, two-side Wilcoxon test and adjusted p-value (pAdj) corrected using the Benjamini–Hochberg method. The cut-off value for the false discovery rate was pAdj > 0.05. Genes with log$_2$ fold change >1 or < -1, pAdj <0.05 and without 0 value from all sample were considered significant. For significance of each GSEA category, significantly regulated gene sets were evaluated with the Kolmogorov-Smirnov statistic. P-values of antibody and qRT-PCR between two groups were calculated using one-tailed Mann-Whitney t-test on GraphPad Prism software (version 9.0.0). A value of *$p$ < 0.05, **$p$ < 0.01, ***$p$ < 0.001, ****$p$ < 0.0001 was considered statistically significant.

**References**

Ai, J., Wang, X., He, X., Zhao, X., Zhang, Y., Jiang, Y., Li, M., Cui, Y., Chen, Y., Qiao, R., et al. (2022). Antibody evasion of SARS-CoV-2 Omicron BA.1, BA.1.1, BA.2, and BA.3 sub-lineages. Cell Host Microbe 30, 1-7. 10.1016/j.c hors.2022.05.001.

Anders, S., Pyl, P.T., and Huber, W. (2015). HTSeq--a Python framework to work with high-throughput sequencing data. Bioinformatics 31, 166-169. 10.1093/bioinformatics/btu638.

Andreano, E., Paciello, I., Piccini, G., Manganaro, N., Pileri, P., Hyseni, I., Leonardi, M., Pantano, E., Abbiento, V., Benincasa, L., et al. (2021). Hybrid immunity improves B cells and antibodies against SARS-CoV-2 variants. Nature 600, 530-535. 10.1038/s41586-021-04117-7.
Andreano, E., and Rappuoli, R. (2021). Immunodominant antibody germlines in COVID-19. J Exp Med 218. 10.1084/jem.20210281.

Arora, P., Zhang, L., Kruger, N., Rocha, C., Sidarovich, A., Schulz, S., Kempf, A., Graichen, L., Moldenhauer, A.S., Cossmann, A., et al. (2022a). SARS-CoV-2 Omicron sublineages show comparable cell entry but differential neutralization by therapeutic antibodies. Cell Host Microbe 30, 1-9. 10.1016/j.chom.2022.04.017.

Arora, P., Zhang, L., Rocha, C., Sidarovich, A., Kempf, A., Schulz, S., Cossmann, A., Manger, B., Baier, E., Tampe, B., et al. (2022b). Comparable neutralisation evasion of SARS-CoV-2 omicron subvariants BA.1, BA.2, and BA.3. Lancet Infect Dis. 10.1016/S1473-3099(22)00224-9.

Bolger, A.M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114-2120. 10.1093/bioinformatics/btu170.

Bolotin, D.A., Poslavsky, S., Davydov, A.N., Frenkel, F.E., Fanchi, L., Zolotareva, O.I., Hemmers, S., Putintseva, E.V., Obraztsova, A.S., Shugay, M., et al. (2017). Antigen receptor repertoire profiling from RNA-seq data. Nat Biotechnol 35, 908-911. 10.1038/nbt.3979.

Bolotin, D.A., Poslavsky, S., Mitrophanov, I., Shugay, M., Mamedov, I.Z., Putintseva, E.V., and Chudakov, D.M. (2015). MiXCR: software for comprehensive adaptive immunity profiling. Nat Methods 12, 380-381. 10.1038/nmeth.3364.

Cao, X. (2021). ISG15 secretion exacerbates inflammation in SARS-CoV-2 infection. Nat Immunol 22, 1360-1362. 10.1038/s41590-021-01056-3.

Cele, S., Jackson, L., Khoury, D.S., Khan, K., Moyo-Gwete, T., Tegally, H., San, J.E., Cromer, D., Scheepers, C., Amoako, D.G., et al. (2022). Omicron extensively but incompletely escapes Pfizer BNT162b2 neutralization. Nature 602, 654-656. 10.1038/s41586-021-04387-1.

Collier, D.A., Ferreira, I., Kotagiri, P., Datir, R.P., Lim, E.Y., Touizer, E., Meng, B., Abdullahi, A., Collaboration, C.-N.B.C.-., Elmer, A., et al. (2021). Age-related immune
response heterogeneity to SARS-CoV-2 vaccine BNT162b2. Nature 596, 417-422. 10.1038/s41586-021-03739-1.

Dejnirattisai, W., Huo, J., Zhou, D., Zahradnik, J., Supasa, P., Liu, C., Duyvesteyn, H.M.E., Ginn, H.M., Mentzer, A.J., Tuekprakhon, A., et al. (2022). SARS-CoV-2 Omicron-B.1.1.529 leads to widespread escape from neutralizing antibody responses. Cell 185, 467-484 e415. 10.1016/j.cell.2021.12.046.

Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., and Gingeras, T.R. (2013). STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29, 15-21. 10.1093/bioinformatics/bts635.

Edara, V.V., Manning, K.E., Ellis, M., Lai, L., Moore, K.M., Foster, S.L., Floyd, K., Davis-Gardner, M.E., Mantus, G., Nyhoff, L.E., et al. (2022). mRNA-1273 and BNT162b2 mRNA vaccines have reduced neutralizing activity against the SARS-CoV-2 omicron variant. Cell Rep Med 3, 100529. 10.1016/j.xcrm.2022.100529.

Gutmann, C., Takov, K., Burnap, S.A., Singh, B., Ali, H., Theofilatos, K., Reed, E., Hasman, M., Nabeebaccus, A., Fish, M., et al. (2021). SARS-CoV-2 RNAemia and proteomic trajectories inform prognostication in COVID-19 patients admitted to intensive care. Nat Commun 12, 3406. 10.1038/s41467-021-23494-1.

He, B., Liu, S., Wang, Y., Xu, M., Cai, W., Liu, J., Bai, W., Ye, S., Ma, Y., Hu, H., et al. (2021). Rapid isolation and immune profiling of SARS-CoV-2 specific memory B cell in convalescent COVID-19 patients via LIBRA-seq. Signal Transduct Target Ther 6, 195. 10.1038/s41392-021-00610-7.

Hoffmann, M., Krüger, N., Schulz, S., Cossmann, A., Rocha, C., Kempf, A., Nehrmeier, I., Graichen, L., Moldenhauer, A.S., Winkler, M.S., et al. (2022). The Omicron variant is highly resistant against antibody-mediated neutralization: Implications for control of the COVID-19 pandemic. Cell 185, 447-456.e411. 10.1016/j.cell.2021.12.032.

Jabeen, A., Ahmad, N., and Raza, K. (2022). Global Gene Expression and Docking Profiling of COVID-19 Infection. Front Genet 13, 870836. 10.3389/fgene.2022.870836.
Knabl, L., Lee, H.K., Wieser, M., Mur, A., Zabernigg, A., Knabl, L., Rauch, S., Bock, M., Schumacher, J., Kaiser, N., et al. (2022). BNT162b2 vaccination enhances interferon-JAK-STAT-regulated antiviral programs in COVID-19 patients infected with the SARS-CoV-2 Beta variant. Communications Medicine 2, 17. 10.1038/s43856-022-00083-x.

Lee, H.K., Knabl, L., Moliva, J.I., Knabl, L., Sr., Werner, A.P., Boyoglu-Barnum, S., Kapferer, S., Pateter, B., Walter, M., Sullivan, N.J., et al. (2022a). mRNA vaccination in octogenarians 15 and 20 months after recovery from COVID-19 elicits robust immune and antibody responses that include Omicron. Cell Rep 39, 110680. 10.1016/j.celrep.2022.110680.

Lee, H.K., Knabl, L., Walter, M., Knabl, L.S., Dai, Y., Füßl, M., Caf, Y., Jeller, C., Knabl, P., Obermoser, M., et al. (2022b). Prior vaccination exceeds prior infection in eliciting innate and humoral immune responses in Omicron infected outpatients. Frontiers in immunology in press.

Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15, 550. 10.1186/s13059-014-0550-8.

Mannar, D., Saville, J.W., Zhu, X., Srivastava, S.S., Berezuk, A.M., Tuttle, K.S., Marquez, A.C., Sekirov, I., and Subramaniam, S. (2022). SARS-CoV-2 Omicron variant: Antibody evasion and cryo-EM structure of spike protein-ACE2 complex. Science 375, 760-764. 10.1126/science.abn7760.

Munnur, D., Teo, Q., Eggermont, D., Lee, H.H.Y., Thery, F., Ho, J., van Leur, S.W., Ng, W.W.S., Siu, L.Y.L., Beling, A., et al. (2021). Altered ISGylation drives aberrant macrophage-dependent immune responses during SARS-CoV-2 infection. Nat Immunol 22, 1416-1427. 10.1038/s41590-021-01035-8.

Risso, D., Ngai, J., Speed, T.P., and Dudoit, S. (2014). Normalization of RNA-seq data using factor analysis of control genes or samples. Nat Biotechnol 32, 896-902. 10.1038/nbt.2931.
Rössler, A., Knabl, L., von Laer, D., and Kimpel, J. (2022). Neutralization Profile after Recovery from SARS-CoV-2 Omicron Infection. N Engl J Med 386, 1764-1766. 10.1056/NEJMc2201607.

Schmidt, F., Muecksch, F., Weisblum, Y., Da Silva, J., Bednarski, E., Cho, A., Wang, Z., Gaebler, C., Caskey, M., Nussenzweig, M.C., et al. (2022). Plasma Neutralization of the SARS-CoV-2 Omicron Variant. N Engl J Med 386, 599-601. 10.1056/NEJMc2119641.

Schurch, N.J., Schofield, P., Gierlinski, M., Cole, C., Sherstnev, A., Singh, V., Wrobel, N., Gharbi, K., Simpson, G.G., Owen-Hughes, T., et al. (2016). Erratum: How many biological replicates are needed in an RNA-seq experiment and which differential expression tool should you use? RNA 22, 1641. 10.1261/rna.058339.116.

Stephenson, E., Reynolds, G., Botting, R.A., Calero-Nieto, F.J., Morgan, M.D., Tuong, Z.K., Bach, K., Sungnak, W., Worlock, K.B., Yoshida, M., et al. (2021). Single-cell multi-omics analysis of the immune response in COVID-19. Nat Med 27, 904-916. 10.1038/s41591-021-01329-2.

Sureshchandra, S., Lewis, S.A., Doratt, B.M., Jankeel, A., Coimbra Ibraim, I., and Messaoudi, I. (2021). Single-cell profiling of T and B cell repertoires following SARS-CoV-2 mRNA vaccine. JCI Insight 6. 10.1172/jci.insight.153201.

Suryawanshi, R.K., Chen, I.P., Ma, T., Syed, A.M., Brazer, N., Saldhi, P., Simoneau, C.R., Ciling, A., Khalid, M.M., Sreekumar, B., et al. (2022). Limited cross-variant immunity from SARS-CoV-2 Omicron without vaccination. Nature. 10.1038/s41586-022-04865-0.

VanBlargan, L.A., Errico, J.M., Halfmann, P.J., Zost, S.J., Crowe, J.E., Jr., Purcell, L.A., Kawaoka, Y., Corti, D., Fremont, D.H., and Diamond, M.S. (2022). An infectious SARS-CoV-2 B.1.1.529 Omicron virus escapes neutralization by therapeutic monoclonal antibodies. Nat Med 28, 490-495. 10.1038/s41591-021-01678-y.

Viana, R., Moyo, S., Amoako, D.G., Tegally, H., Scheepers, C., Althaus, C.L., Anyaneji, U.J., Bester, P.A., Boni, M.F., Chand, M., et al. (2022). Rapid epidemic expansion of the
SARS-CoV-2 Omicron variant in southern Africa. Nature 603, 679-686. 10.1038/s41586-022-04411-y.

Wickham, H. (2009). Ggplot2 : elegant graphics for data analysis (Springer).

Yan, Q., He, P., Huang, X., Luo, K., Zhang, Y., Yi, H., Wang, Q., Li, F., Hou, R., Fan, X., et al. (2021). Germline IGHV3-53-encoded RBD-targeting neutralizing antibodies are commonly present in the antibody repertoires of COVID-19 patients. Emerg Microbes Infect 10, 1097-1111. 10.1080/22221751.2021.1925594.

Yu, J., Collier, A.Y., Rowe, M., Mardas, F., Ventura, J.D., Wan, H., Miller, J., Powers, O., Chung, B., Siamatu, M., et al. (2022). Neutralization of the SARS-CoV-2 Omicron BA.1 and BA.2 Variants. N Engl J Med 386, 1579-1580. 10.1056/NEJMc2201849.

Yuan, M., Liu, H., Wu, N.C., Lee, C.D., Zhu, X., Zhao, F., Huang, D., Yu, W., Hua, Y., Tien, H., et al. (2020). Structural basis of a shared antibody response to SARS-CoV-2. Science 369, 1119-1123. 10.1126/science.abd2321.

Zhang, Q., Ju, B., Ge, J., Chan, J.F., Cheng, L., Wang, R., Huang, W., Fang, M., Chen, P., Zhou, B., et al. (2021a). Potent and protective IGHV3-53/3-66 public antibodies and their shared escape mutant on the spike of SARS-CoV-2. Nat Commun 12, 4210. 10.1038/s41467-021-24514-w.

Zhang, Y., Guo, X., Li, C., Kou, Z., Lin, L., Yao, M., Pang, B., Zhang, X., Duan, Q., Tian, X., et al. (2021b). Transcriptome Analysis of Peripheral Blood Mononuclear Cells in SARS-CoV-2 Naive and Recovered Individuals Vaccinated With Inactivated Vaccine. Front Cell Infect Microbiol 11, 821828. 10.3389/fcimb.2021.821828.

Zhao, Y., Li, M.C., Konate, M.M., Chen, L., Das, B., Karlovich, C., Williams, P.M., Evrard, Y.A., Doroshow, J.H., and McShane, L.M. (2021). TPM, FPKM, or Normalized Counts? A Comparative Study of Quantification Measures for the Analysis of RNA-seq Data from the NCI Patient-Derived Models Repository. J Transl Med 19, 269. 10.1186/s12967-021-02936-w.
**Figure legends**

**Figure 1. Antibody analysis.** (A) Plasma IgG antibody binding to the SARS-CoV-2 RBD (spike) of the ancestral (WA) variant and Omicron BA.1 sublineage. (B) Plasma IgG antibody binding to the SARS-CoV-2 RBD (spike) of different variants. Plasma from unvaccinated Omicron patients with and without prior infection experience was analyzed. WA, authentic SARS-CoV-2 strain. (C-D) Neutralization response to virus spike protein of the ancestral strain and Omicron variants (C) as well as the SARS-CoV-2 RBD (spike) of former variants (D). p-value between two groups is from one-tailed Mann-Whitney t-test. Percentage of samples with zero inhibition activity for each group is shown above the X axis. Asterisks indicate significance between groups. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Line at median, dotted line at 90%.

**Figure 2. Immune transcriptomes following Omicron infection.** (A) Volcano plot of differentially expressed genes (DEGs) in unvaccinated patients within 2-3 days after infection with Omicron sublineages BA.1 or BA.2. The patients had no known prior infection with other SARS-CoV-2 variants. (B) GSEA categories of genes expressed at significantly higher levels in the BA.1 or BA.2 infection group. X axis denotes statistical significance as measured by minus logarithm of FDR q-values. Y axis ranked the terms by q values. (C) Bar plots depicting the fold change of specific mRNA levels between the BA.1 and BA.2 groups. Red bars show genes that are higher in the BA.1 group compared to BA.2 and enriched in neutrophil degranulation and innate immune system. Blue bars indicate genes that are higher in the BA.2 group compared to BA.1 and enriched in complement pathways. (D) Volcano plot of DEGs in unvaccinated patients, with and without prior SARS-CoV-2 exposure, 2-3 days after infection with the Omicron BA.2 sublineage. (E) GSEA categories of genes expressed at significantly higher levels in the BA.2 groups with and without prior SARS-CoV-2 infection. (F) Bar plots showing the fold change of specific mRNA levels between BA.2 groups with and without prior SARS-CoV-2 with earlier variants. Blue bars present genes that are higher in the no prior infection group compared to the prior infection group and are enriched in innate immune responses.
**Figure 3. SARS-CoV-2-RBD-specific B-cell memory in Omicron patients.** (A) The number of total immunoglobulin alleles in each group at days 13-14. (B) Box plots show the frequency (%) of IGHV usage in the BA.1 and BA.2 groups with no prior infection. Alleles previously identified in neutralizing antibodies to COVID-19 infection are marked in red. *p*-value between two groups is from one-tailed Mann-Whitney t-test. *p* < 0.05. Median, middle bar inside the box; IQR, 50% of the data; whiskers, 1.5 times the IQR.
Table 1. Characteristics of Omicron study population.

|                                | No prior infection | Prior infection         | Chi-Square (p-value) |
|--------------------------------|--------------------|-------------------------|----------------------|
|                                | BA.1               | BA.2                    | BA.1                | BA.2            |
| N                              | 40                 | 10                      | 21                  | 3               |
| Age (years), median (range)    | 42 (9-83)          | 40 (13-65)              | 33 (10-63)          | 33 (28-43)      | 1.3 (0.73)    |
| Gender                         |                    |                         |                      |                  |
| Female                         | 31 (78%)           | 5 (50%)                 | 11 (52%)            | 2 (67%)         | 22.22 (0.00006) |
| Male                           | 9 (22%)            | 5 (50%)                 | 10 (48%)            | 1 (33%)         |
| Medical condition              |                    |                         |                      |                  |
| Auto-immune Disease            | 1                  | 0                       | 2                    | 0               |
| Chronic Heart Disease          | 1                  | 0                       | 0                    | 0               |
| Chronic Pulmonary Disease      | 2                  | 0                       | 0                    | 0               |
| Dementia                       | 1                  | 0                       | 0                    | 0               |
| Diabetes                       | 1                  | 0                       | 0                    | 0               |
| Gout                           | 1                  | 0                       | 0                    | 0               |
| Hypertension                   | 4                  | 0                       | 0                    | 0               |
| Hypothyroid                    | 7                  | 3                       | 3                    | 1               |
| Kidney Disease                 | 1                  | 0                       | 0                    | 0               |
| Multiple Sclerosis             | 1                  | 0                       | 0                    | 0               |
| S/P Cancer                     | 1                  | 0                       | 0                    | 0               |
| COVID-19 disease severity      |                    |                         |                      |                  |
| Asymptomatic                   | 3 (7.5%)           | 0                       | 0                    | 0               | 46.61 (<0.00001) |
| Mild                           | 15 (37.5%)         | 6 (60%)                 | 13 (62%)            | 3 (100%)        |
| Mild with SOB*                 | 13 (32.5%)         | 4 (40%)                 | 6 (29%)             | 0               |
| Moderate                       | 7 (17.5%)          | 0                       | 2 (10%)             | 0               |
| Severe                         | 2 (5%)             | 0                       | 0                    | 0               |
| Days from positive PCR test to sampling, median (range) | | | | |
| 1st RNA samples                | 2 (0-4)            | 3 (0-4)                 | 2 (0-5)             | 3 (2-3)         |
| 2nd RNA samples                | 14 (7-15)          | 14 (12-17)              | 13 (7-18)           | 16 (12-19)      | 0.21 (0.98)   |
| Serum samples                  | 14 (7-21)          | 14 (7-17)               | 13 (7-18)           | 14 (7-19)       |

*SOB, shortness of breath.
Highlights

- Reduced immune response in Omicron BA.2 patients without previous history of COVID-19
- Elevated immune response in BA.1 and BA.2 patients with previous history of COVID-19
- Distinct antibody creation and immune gene expression between BA.1 and BA.2 patients