

Supplementary Methods

Single B cell RT-PCR and antibody cloning

Single memory B cells isolated from PBMCs of vaccinated convalescent donors were cloned as previously described (1, 2). Briefly, one-step RT-PCR was performed on sorted single memory B cells with a gene-specific primer mix, followed by nested PCR amplifications and sequencing using heavy chain and light chain-specific primers. Cloning PCR was then performed using heavy chain and light chain-specific primers containing specific restriction enzyme cutting sites (heavy chain, 5'-AgeI/3'-Sall; kappa chain, 5'-AgeI/3'-BsiWI). The PCR products were purified and cloned into the backbone of human Igy1 or Igk expression vectors using T4 ligase (NEB). The constructed plasmids containing paired heavy and light chain expression cassettes were co-transfected into HEK293T cells grown in 6-well plates. Antigen-specific ELISA and pseudovirus-based neutralization assays were used to analyze the binding capacity to SARS-CoV-2 spike trimers and different domains, as well as the neutralization capacity of transfected culture supernatants, respectively.

Genetic analysis of the BCR repertoire

Heavy chain and light chain germline assignment, framework region annotation, determination of somatic hypermutation (SHM) levels (in nucleotides) and CDR loop lengths (in amino acids) were performed with the aid of the NCBI IgBlast tool (<https://www.ncbi.nlm.nih.gov/igblast/>) as previously described (1). Sequences were aligned using Clustal W in SnapGene 5.2. An antibody clonal family was defined as a set of antibodies encoded by the same immunoglobulin-heavy variable (IGHV) gene and joining (IGHJ) gene with complementarity-determining region (CDR) H3 sequence similarity above 85% (3). Phylogenetic trees were generated by MEGA 11.

Antibody production and purification

Antibodies were produced and purified as previously described (1). Paired heavy and light chain plasmids were co-transfected into Expi293FTM cells grown in the 250 mL flask for 5-7 d. Antibodies produced from cell culture supernatants were purified immediately by affinity chromatography using recombinant Protein G-Agarose (Thermo Scientific) according to the

manufacturer's instructions to purify IgG. The purified antibodies were concentrated by an Amicon ultracentrifuge filter device (molecular weight cut-off 10 kDa; Millipore) to a volume of 0.2-0.5 mL in PBS (Life Technologies) then stored at 4 °C for further characterization.

Epitope mapping by ELISA

Competition ELISA was performed as previously described (4). Purified mAbs were biotin-labeled using One-Step Antibody Biotinylation Kit (Miltenyi Biotec) following the manufacturer's recommendations and purified using the Amicon ultracentrifuge filter device (10 kDa, Millipore). Competitor antibodies (50 µL) were added into trimeric spike-precoated ELISA plates and incubated at 37 °C for 1 hour. Wells were then incubated with serially diluted biotinylated antibodies (50 µL) at a concentration that achieves an OD450 reading of 2-3 in the absence of competitor antibodies. Plates were incubated at 37 °C for 1 hour, and 50 µL of 500-fold diluted Avidin-HRP (ThermoFisher Scientific) was added into each well and incubated for another 1 hour at 37 °C. The plates were washed with PBST between each of the previous steps and developed afterwards with TMB substrate (SIGMA) and OD450 was measured by SkanIt RE6.1 with VARIOSKAN Lux (Thermo Scientific) after the reaction was stopped. The relative binding of biotinylated antibodies to the spike in the presence of competitors was normalized by comparing to competitor-free controls (PBS containing 4% skim milk). Relative binding curve and the area under curve (AUC) were generated by GraphPad Prism 9.4.1.

IgG digestion

Fab fragments of ZCP3B4, ZCP4C9, ZCP4D5-1 and CUP2G3 were produced by digestion with Pierce™ Fab Preparation Kit (Thermo). The prepared IgG sample (4 mg) was added to the spin column tube containing the equilibrated Immobilized Papain. Incubate The digestion reaction was incubated at 37°C for 5 h with constant mixing. The resulting Fabs were purified from the cleaved Fc domain by affinity chromatography using protein A. All Fabs were buffer-exchanged into PBS, pH 7.4 prior to cryo-EM.

Quantification of the infectious virus

The plaque assay as we described previously (5). Briefly, serial 10-fold dilutions of each tissue

homogenate were inoculated in a Vero-E6 cell monolayer in quadruplicate and cultured in DMEM supplemented with 1% FBS and penicillin/streptomycin. The plates were observed for cytopathic effects at day 4. Plaque-forming units (PFUs) were calculated by the number of plaques multiplied by the dilution factor and expressed as PFU/mL of tissue homogenate.

Histopathological analysis and immunofluorescence (IF) staining

Hematoxylin and Eosin (H&E) as well as immunofluorescence (IF) staining was performed as previously described (5). Lung sections were collected, fixed in the 4% formaldehyde solution and embedded in paraffin for H&E staining. The whole tissue sections after H&E staining were scanned and analyzed using the Akoya Vectra Polaris™ Automated Quantitative Pathology Imaging System. The IF staining was conducted for identification and localization of SARS-CoV-2 nucleocapsid protein (NP) using a rabbit anti-SARS-CoV-2-N antibody (1:5000) together with Alexa Fluor 488-conjugated goat anti-rabbit IgG antibody (1:1000 Life Technologies). The images of the lung tissue section were captured using the Carl Zeiss LSM 900 confocal microscope and analyzed using the ZEN 3.3 software (Blue edition).

Quantification of relative amounts of Omicron variants in specimens

The next-generation sequencing was performed as previously described (6). The extracted RNA from tissues was reversed transcribed into cDNA using SuperScript™ IV reverse transcriptase (Thermo Fisher Scientific, Waltham, MA). The cDNA was then amplified using ARTIC network nCoV-2019 primers v3 (Integrated DNA Technologies, Coralville, IA) using Q5® High-Fidelity DNA Polymerase (New England Biolabs, Ipswich, Massachusetts, United States) as we described previously with minor modifications and only the spike gene region was amplified (7). The PCR amplicons were purified using AMPure XP beads (Beckman Coulter, Brea, CA) and quantified by Qubit (Thermo Fisher Scientific). The Illumina libraries were prepared using the KAPA HyperPrep Kit (Roche Applied Science, Penzberg, Germany) using 100 ng as input following the manufacture protocol with double-sided size selection to select adapter ligated libraries with the size range 300-750 bp. PCR was performed for library enrichment followed by purification using AMPure XP beads. The enriched libraries were validated using gel electrophoresis, Qubit and qPCR for quality control analysis. The libraries

were denatured and diluted to optimal concentration prior sequencing on the Illumina MiSeq System using the MiSeq Reagent Kit v3 (600-cycle) (Illumina, San Diego, CA) for paired-end sequencing (PE301). The output fastq files were subjected to adapter removal using FASTP (8). The pair end reads were then combined into one read using Paired-End reAd merger (PEAR). Reads with mean Q score less than 20 and merged length shorter than 200 were removed. The first and last 30 bp of each merged reads were trimmed to remove PCR primers. Then, the reads were mapped to NC_045512.2 using BWA MEM. Finally, base compositions from representative positions for each lineage were extracted using the Pysamstats library. Positions with coverage less than 250 were not included in the calculation. The ratios between variants were calculated based on the ratio of reads containing the spike mutations and deletion (G16935A, A22893C, T22942A, C28312T, T21810C, T22200A, C22664A, T22942G, A21993-, G22599C, T22942G and A28330G). The ratios of the variants obtained from different mutations were averaged to produce the result.

References

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