Methods

Sample collection

Serum samples were collected from study participants on the day of each vaccine dose, and 2 weeks after. Unstimulated whole saliva was self-collected using image instructions at the same time points as the serum samples were taken, thereafter placed at +4°C and stored at -80°C within same day. Prior to analysis, saliva samples were thawed at 4°C and centrifuged at 400 x g for one minute at 4°C, and the supernatant was used for the antibody measurement.

SARS-CoV-2 Spike binding antibodies and pseudo-neutralizing test

Serum samples were analyzed with Elecsys® anti-SARS-CoV-2 S immunoassay (Roche Diagnostics) to determine total Ab levels against Spike receptor-binding domain protein (RBD) and Nucleocapsid protein (positive cut-off >0.8 U/mL for RBD; >1.0 for Nucleocapsid). Both serum and saliva samples were then further analyzed with V-PLEX SARS-CoV-2, Panels (Meso Scale Diagnostics, Maryland, USA) for IgA (Panel 24), IgG (Panel 25) and ACE2 blocking (Panel 25) according to the manufacturer's instruction at the SciLifeLab Affinity Proteomics Unit (Uppsala, Sweden). Spike proteins analyzed were from Wu-Hu1 (wild-type) strain, Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1); Delta (B.1.617.2; AY.4) and Omicron BA.1 (B.1.1.529;BA.1).

For binding antibody measurement, samples were diluted 1:100 (saliva) or 1:50 000 (serum) and detected using the SULFO-TAG conjugated mouse monoclonal anti-human IgA or IgG antibody. For inhibition of ACE2-Spike binding, the samples were diluted 1:2 (saliva) or 1:100 (serum) to allow competition with the recombinant human ACE2 conjugated with SULFO-TAG for binding to the respective Spike antigen. The plates were analyzed on a MESO QuickPlex SQ 120 instrument, an electrochemiluminescence reader measuring the light emitted from the SULFO-TAG. Results from the assays are reported in AU/ml and derived from back-fitting the measured signals for samples to a calibration curve generated for each plate. For salivary IgG and IgA, the spike-specific Ab levels were related to total concentrations of IgA and IgG in each sample, quantified (pg/ml) using Isotyping Panel 1 Human/NHP Kit (Meso Scale Diagnostics) according to the manufacturer´s instructions (sample dilution IgA 1:10 000, IgG 1:1000). Ratios were multiplied by 10^7.

The cut-off levels for binding serum Abs were provided by the manufacturer. To estimate the pseudo-neutralization capacity of saliva samples, the intensity signals from the ACE2 assay

were compared to mean plus 3x standard deviation of the intensity signals of 27 negative control saliva samples (pre-pandemic and seronegative saliva from 2020).

T cell assays

Activation-induced marker (AIM) assay was carried out as previously described (Gao et al, 2022). In brief, cryopreserved PBMCs were thawed and resuspended in complete media. After resting, cells were incubated with an overlapping peptide pool of the entire Spike protein sequence of wild-type or BA.1 of SARS-CoV-2 at a final concentration of 0.5 ug/ml. After 12 hours, cells were washed in PBS and stained with Aqua (Live/Dead staining) and anti-CCR7-APC-Cy7 (BioLegend) for 10 min at 37°C. Additional surface stains with anti-154 (CD40L)-BV421, anti-CD137(4-1BB)-PE-Cy7, anti-CD14-BV510, anti-CD19-BV510, antiCD45RA-BV570, anti-CD69-BV650 (BioLegend); anti-CD3-BUV805, anti-CD8-BUV395 and anti-CD4-BUV496 (BD Biosciences) for 30 min at room temperature, together with Brilliant Stain Buffer Plus (BD Biosciences). Stained cells were fixed in PBS containing 1% paraformaldehyde (PFA; Biotium) and acquired using a FACS Symphony A5 (BD Biosciences). Results are shown as frequency (%) of Spike-specific cells after subtraction of the paired frequency of AIM+ cells stimulated with DMSO. Negative values and values below 0.001 were set to a specified lower limit of quantification set at 0.001 to allow graphing with a logarithmic scale with a gating strategy as described earlier (Blixt et al. 2022).

Statistical analyses

All analyses were pre-specified, and data is summarized using descriptive statistics such as counts, percentages, medians, and ranges. Categorical variables are presented as cross-tabulations, and distributional differences were tested using Fisher's exact test. Comparisons between groups or time points were performed by unpaired, non-parametric Mann-Whitney test. Correlation analysis was done using non-parametric Spearman rank correlation. P-values <0.05 were considered significant. Statistical tests were performed in Prism v.9 (GraphPad Software Inc.) and R v.4.0.2.