Supplementary material and methods

Anti-RBD ELISA

Serum samples were collected at various time points using venipuncture and home-based fingerprick sets to determine the presence of SARS-CoV-2 antibodies using three in-house developed anti-RBD IgG ELISA.[1,2] First, anti-RBD IgG levels were measured with a quantitative ELISA and expressed as arbitrary units per milliliter (AU/mL). Serum was compared to a serially diluted calibrator consisting of pooled convalescent plasma. Seroconversion was defined as antibody titer >4 AU/mL, with a 99%-specificity in pre-pandemic sera. Second, individuals with prior SARS-CoV-2 infection before vaccination were identified using a semi-quantitative total antibody bridging ELISA. This ELISA demonstrated increased sensitivity in very low antibody ranges (98.1% sensitivity and 99.5%-specificity) compared to the anti-RBD IgG ELISA. Lastly, a semi-quantitative total antibody bridging ELISA against Nucleocapsid antibodies was used to detect SARS-CoV-2 infection after the first vaccination.

Whole blood flow cytometry

Fresh whole blood was stained with two antibody panels consisting of 38 unique human immune markers (table S2), as previously described.[3–5] In short, fresh whole blood was stained with both antibody panels for 30 minutes at room temperature (RT), treated with BD FACS Lysing solution (BD Biosciences) for 10 minutes at RT to lyse erythrocytes, followed by wash and fixation of cells with 1% PFA for 20 minutes at 4°C. Samples were resuspended in PBS with 0.5% bovine serum albumin and 2 mM ethylenediaminetetraacetic acid. Count bright Plus Absolute Counting Beads (ThermoFisher) were added to calculate absolute cell numbers. Samples were acquired on a BD FACSymphony (BD).

Computational flow cytometry analysis of whole blood

Computational flow cytometry analysis of fresh whole blood has previously been described.[3–5] Briefly, data analysis was performed using the Spectre R package.[6] First, single cells were gated in FlowJo v10 software (FlowJo), and anomalies were removed using the flowAI R package.[7] A hyperbolic arcsin (arcsinh) transformation was applied to reduce noise during clustering, also data points below the detection limit were compressed. Next, samples were corrected for batch effects using reciprocal principle component analysis (rPCA) from the Seurat toolkit for cellular genomics,[8] expression levels from each batch were compared to a 'reference' batch. High-dimensional FlowSOM analysis was used to visualize different immune subsets. Expression of CD19, CD20, CD10, CD38, CD27, CD138, CD11c was used for B cell cluster annotation; CD3, CD4, CXCR5, CXCR3, CCR6, CCR4, CD27, CD95 and CD45RA for CD4 T cell cluster annotation and CD3, CD8, CD45RA, CD27 and CD95 for CD8 T cell annotation (Supplementary table 3). Additional markers in the second panel were used to study the activation status of T cells, including CD38, HLADR, TIGIT, CD137, CD40L, PD1, TIM3, CTLA4, and ICOS. As the ICOS stain did not yield positive results, it was excluded from further analyses.

B cell spectral flow cytometry staining

Antigen probe design, purification, and antigen-specific B cell staining were performed as previously described.[9–12] In short, PBMCs were thawed in IMDM (Lonza) with 10% FCS (Bodinco BV). 10 x 10^6 PBMCs were depleted of CD3+ cells using EasySep™ Human CD3 Positive Selection Kit II (StemCell Technologies) according to the manufacturer's protocol.

To stain antigen-specific B cells, biotinylated protein antigens SARS-CoV-2 Spike-2P (prefusion stabilized Spike trimer), SARS-CoV-2 RBD, Nucleocapsid protein (NCP), influenza hemagglutinin (HA; H1N1pdm2009), prefusion stabilized glycoprotein from respiratory syncytial virus (RSV; DS-Cav1) and Tetanus Toxoid (TT; Vcar-Lsx003) were individually multimerized with fluorochrome-conjugated streptavidin in a 2:1 molar ratio at 4°C for 1 hour on a shaker. Next, 10% D-biotin (GeneCopoeia) was added to each multimerized protein antigen and incubated at 4°C for at least 30 minutes to reduce cross-reactivity among biotinylated protein antigens. All biotinylated antigens were conjugated to two fluorochromes, except RBD was conjugated to a single fluorochrome as it is part of Spike (Supplementary table 4). A 31-color spectral cytometry panel, including the six biotinylated antigens, was designed to immunophenotype antigen-specific B cells (Supplementary table 5). Samples were first stained with Live/Dead Fixable Blue Stain Kit (Invitrogen) in PBS for 30min at 4°C. After washing with staining buffer containing 1% BSA and 1 mM EDTA in phosphate-buffered saline, cells were stained with the above mentioned spectral cytometry panel including the protein antigens for 30min at 4°C. Cells were fixed with cold paraformaldehyde 1% for 10min at RT and washed again twice with washing buffer. Data were acquired on Cytek Aurora 5L using SpectroFlo® software (Cytek Biosciences).

Computational flow cytometry B cell data pre-processing

After spectral unmixing (SpectroFlo v3.0.1, Cytek), FCS files were loaded into OMIQ software from Dotmatics. Data were transformed using arcinh. Initial gating was performed to select for single, live, CD19+, DUMP and autofluorescent negative cells (DUMP included CD3, CD4, CD14, CD56) (Figure S3A). PeacoQC was run to detect and remove flow cytometry anomalies in both signal acquisition and dynamic range.[13] To exclude batch effects, all data were normalized using Cytonorm for 8 markers (CD20, CD38, CD19, CD24, HLA-DR, CD45RB, IgD, IgM) using a reference sample which was stained and measured in each batch to control for signal variation, as described before.[14] Subsequently, antigen-specific B cells were gated based on their specific combination of fluorochrome-conjugated streptavidin, and were negatively gated for all other fluorochrome combinations; RBD-positive cells were gated from Spike-positive cells (Figure S3B). For downstream analysis, data were subsampled to include only antigen-specific B cells.

Computational flow cytometry dimensionality reduction and FlowSOM clustering

To obtain sufficient input data for UMAP visualization and FlowSOM clustering, we included antigen-specific B cell data from 104 participants from the T2B study cohort.[15] This cohort included IMID patients using different ISPs, such as MTX and TNF inhibitors, IMID patients without ISP, and HCs. For UMAP visualization and FlowSOM clustering (with dimensions xdim=16 and ydim=16), 12 lineage markers (CD20, CD21, CD27, CD138, CD38, CD24, CD45RB, CD11c, IgM, IgA, IgG, IgD) were given as input. Consensus meta clustering was then run to generate k = 50 meta clusters, which were manually merged into 16 populations based on biological relevance. The 16 populations were annotated based on UMAP visualization and heatmap analysis of the median marker expression between the clusters (annotations are listed in supplementary table 6). Further downstream analyses on Spike- and RBD-specific B cells were performed using only data from study participants included in the current paper.

Activation-induced marker (AIM) assay

PBMCs were thawed in IMDM with 5% FCS, 1% penicillin/streptavidin and 0.1% DNAse, centrifuged, and resuspended in IMDM with 5% FCS and 5% HS. PBMCs were plated at 2 x 10^6 cells per well and stimulated with a Spike 1 and Spike 2 protein peptide pool (15-mer with 11aa overlap, JPT-Innovative Peptide-Solutions PM-WCPV-S-2; at a final concentration of 1 ug/mL) for 18 hours at 37ºC. Brefeldin A (ThermoFisher, diluted 1:1000) was added two hours after Spike peptide stimulation. DMSO and anti-CD3/CD28 (ThermoFisher) stimulation were used as a negative and positive control, respectively. The next day, PBMCs were stained with a panel consisting of 23 fluorescent conjugated antibodies (Supplementary table 7). First, PBMCs were stained with fluorescently labeled antibodies for extracellular staining for 30 minutes at RT in the dark. Next, PBMCs were washed and fixated (Foxp3 staining buffer set, eBioscience) for 30 minutes at 4ºC. Subsequently, PBMCs were washed and stained for 30 minutes at 4ºC in the dark with fluorescently labeled antibodies for intracellular staining, which were diluted in Permeabilization buffer (ThermoFisher). Cells were washed and acquired on a FACSymphony (BD). Rainbow beads (BD) were used as a reference to correct voltage settings during each acquisition. FlowAI R-plugin was used for data quality control; this included anomaly detection via flow rate check (timestep 1/10 second), signal acquisition (threshold: 1300) and dynamic range (upper- and lower limit). Data were manually analyzed using FlowJo v10 software (FlowJo). DMSO background was subtracted from Spike-induced CD4 T cell percentages during analysis.

Statistical analysis

All statistical analyses were performed using Rstudio (version 4.1.1). Statistical significance was determined using the Wilcoxon rank sum test for unpaired comparisons and the Wilcoxon signed-rank test for paired data. Bonferroni-Holm's method of multiple comparisons was used to correct *p value*s in case of multiple testing. Spearman's correlation coefficient was used to assess correlations. *P values* lower than 0.05 were considered statistically significant.

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