Supporting information

Extended methods

Participants and samples

The study was conducted at the Sahlgrenska University Hospital and the University of Gothenburg, Sweden. Study patients (n=44) were otherwise healthy hospital workers who provided written informed consent. Eighty-one samples from these donors were analyzed between December 2020 and December 2022. Ethics approval was granted by the Swedish Ethical Review Authority (permits no. 2020-03276, 2021-00374, and 2021-00539) and by the Swedish Medical Products Agency (EudraCT no. 2021-000349-42). Peripheral blood (8-24mL) was collected in serum tubes and lithium-heparin tubes (Becton Dickinson, Stockholm, Sweden). Thirty patients previously diagnosed with COVID-19, confirmed by SARS-CoV-2 RNA RT-qPCR, contributed 56 whole blood samples. One patient had severe COVID-19 requiring hospitalization; the remaining 29 patients had mild infection. Median age was 38 (range 25-66) years and 77% were female. Fourteen participants contributed with 1 sample, 9 with 2 samples, 4 with 3 samples and 3 with 4 samples at different timepoints after infection that were analyzed for presence of nucleocapsid specific T cells. Twenty-three previously infected patients contributed one sample prior to vaccination that was analyzed for presence of spike 1-specific T cells. Three participants were infected during the study and were thereafter analyzed together with infected patients. Median days from verified infection to sampling was 267 (range 25-629) days. Uninfected control subjects (n=17) were defined by lack of PCR-verified COVID-19 and seronegativity for IgG against the receptor binding domain of spike 1 at baseline sampling prior to vaccination. Ten controls contributed with 1 sample, 6 with 2 samples and 1 with 3 samples for analysis of nucleocapside specific T cells. Eleven controls

contributed one sample each prior to vaccination for analysis of spike-1-specific T cells. Thirteen (76%) of control subjects were female with median age 55 (range 25-70) years.

Serum IgG against the receptor-binding domain spike 1

To exclude prior COVID-19 among control subjects, serum samples were analyzed for antibodies against the receptor-binding domain of SARS-CoV-2 spike 1 using the chemiluminescent microparticle immunoassay Abbott Alinity SARS-CoV-2 IgG II Quant (Abbott, Abbott Park, IL, USA).

Assay of antigen-specific cytokine release in whole blood samples

One ml of peripheral venous lithium-heparinized blood was incubated with nucleocapsid or spike 1 protein peptides at 1µg/ml/peptide in conical 10 ml tubes (Sarstedt AB, Helsingborg, Sweden. Each sample was stimulated with 15-mer peptides with 11-amino acid overlap spanning the complete sequence of the SARS-CoV-2 nucleocapsid phosphoprotein (aa 1-419, 102 peptides) (NC; 130-126-699, Miltenyi Biotec, Lund, Sweden), the *N*-terminal S1 domain (aa 1-692, 170 peptides) of the SARS-CoV-2 spike 1 surface glycoprotein (S1; 130-127-041, Miltenyi Biotec) or was left unstimulated. After 48 hours of incubation at 37º and 5% CO_2 samples were centrifuged at 1500 rpm for 5 minutes. Plasma supernatants were collected and stored at -80ºC until analysis for cytokine content. Only unvaccinated participants were included in analysis of spike 1 peptide-induced cytokines.

Detection of SARS-CoV-2-induced cytokines in plasma supernatants

Levels of IFN-γ, interleukin-2 (IL-2), IL-4, IL-12p70 (IL-12), IL-13 and IL-17A were measured in the plasma supernatants from SARS-CoV peptide-stimulated and unstimulated whole blood using the FirePlex®-96 Key Cytokines Immunoassay panel (ab243549 and ab285173, Abcam) according to the manufacturer's instructions. These assays measure cytokines with minimal cross-reactivity[1]. Plasma was diluted 1:3 or 1:4 in Human Assay Diluent (Abcam) and samples were acquired in Run Buffer III (Abcam) on a five laser BD LSRFortessa (BD Biosciences), and analyzed using Fireplex Analysis Workbench (Abcam). Cytokine levels in stimulated samples are presented with levels in unstimulated samples subtracted, thus reflecting cytokines induced by SARS-CoV-2-peptides.

The levels of IL-21 in plasma supernatant from unstimulated, NC- and S1-stimulated whole blood were determined by IL-21 ELISA (DY8879-05, R&D Systems) according to manufacturer's instructions. Plasma was diluted 1:2 in PBS containing 1%BSA and 10% rat serum (Stemcell Technologies) to minimize unspecific binding. Optical density was measured at 450 nm and 570 nm using a FLUOstar Omega plate reader (BMG, Ortenberg, Germany). Results are presented as peptide-induced IL-21 obtained by subtracting levels of IL-21 in unstimulated samples from levels in peptide-stimulated samples.

Data analysis and statistics

Mean differences between SARS-CoV-2 peptide-induced cytokine production in uninfected control subjects, patients with samples available <70 days after infection and patients with samples available 70-629 days after infection were compared using permutation tests. This test is similar to the Fisher-Pitman test but in addition accounts for repeated measures. Correlations between SARS-CoV-2-induced cytokine levels were calculated using mean concentrations per

time phase when repeated measures were available. The impact of time after infection on SARS-CoV-2 peptide-induced cytokines was assessed using linear regression or linear mixed-effects models, if repeated measures were present. Model assumptions were examined with residual plots. P-values are two-sided throughout. Statistical analyses were performed using IBM SPSS 28.0 and R 4.0.5 and figures were prepared using GraphPad Prism version 9.3.1.

Data availability

The Supporting information contains a complete set of deidentified primary data showing donor characteristics, SARS-CoV-2-specific serology, SARS-CoV-2 peptide-induced cytokines and background cytokine levels in unstimulated samples at different time points after infection.

References

1. Abcam. (https://www.abcam.com/kits/fireplex-immunoassays-validation-and-benchmarking, 2021).