nature portfolio

Corresponding author(s): Sophie Trouillet-Assant, Thierry Walzer, Jacqueline Marvel, Thierry Defrance, Stéphane Paul, François-Loïc Cosset

Last updated by author(s): Sep 30, 2021

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Clinical data were recorded by a trained Clinical Research Associate using the Clinsight software (version _ Csonline 7.5.720.1). Flow Cytometry data were collected with BD FACSDIVA v8.01 or SpectroFlo V2.2.0 (Cytek)
Data analysis	Statistical analysis were performed using GraphPad Prism version 8. Flow Cytometry data were analyzed with FlowJo Software (10.6.1, FlowJo LLC, BD Life Sciences), GLM in R 4.1.0

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The data that support the findings of this study are included in the “Source data” file available online.
Viral sequences are available on GISAID (GISAID accession numbers: EPI_ISL_1707038 19A (B.38) ; EPI_ISL_1707039 Alpha (B.1.1.7) ; EPI_ISL_768828 Beta (B.1.351) ; EPI_ISL_1359892 Gamma (P.1) ; EPI_ISL_1904989 ; Delta (B.1.617.2).

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	We did not perform sample size calculations as we did not know what to expect in terms of difference for the different measurements. We tried to analyze 30 patients in each group for all immunological measurements, as, based on our experience, it was sufficient to reach good statistics for most of the measurements, given that we minimize variability by selecting patients without comorbidity with similar age range, to avoid batch effects patients from each group (ie homologous vs heterologous) were included in each experiment and the number of experiments performed to test all samples was reduced as much as technically feasible.
Data exclusions	In the T cell experiments in Figure 3G-H we had to exclude several samples in both groups that were all analyzed on the same day. Indeed, for this particular day of experiment, the thawing step was not performed properly and cells died. Because only one PBMC sample was available for each donor, we could not repeat the experiments for the corresponding individuals. No other data were exluded in this study.
Replication	For all neutralization experiments two to three replicates were performed to ensure reproducibility. For commercial Elisa kits, internal quality controls ensure reproducibility. Attempts to replicate Elisa and neutralization experiments were all successful. For flow cytometry experiments, each patient was analyzed once. Samples were run on consecutive days, and the results were consistent over the different days, ensuring the validity of the measurements.
Randomization	Participants were health care workers. The participation of the study did not modify the vaccination schedule of each participant. the intervention on participants was limited to blood sampling. They were allocated in each group on the basis of their vaccination schedule (ie homologous vs heterologous). We recruited patients after the priming step, not before (we therefore did not decide which patients would receive each vaccine). The choice of the prime vaccine was only driven by the availability (both vaccines were approved and given without preference). For immunological assays, samples were blinded and randomized.
Blinding	For all experiments in Figures 2 and 3, we used a blinded strategy to perform immunolgical and serological analyses: Each sample was labelled with the reference (not the name) of the patient, but not the identity of the group (ie heterologous vs homologous vaccination) and the identity was only revealed at the analysis step, after blinded data acquisition.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Pseudovirus neutralization assays: Anti-spike SARS-CoV-2 RBD (Sino Biologicals # 40150-V08B2) 1/100 anti-gp70 RD114 (ViroMed Biosafety Labs,#RD114) 1/100 Elisas Recombinant protein Spike S1-His: SARS-CoV-2 (2019-nCoV) -Cat: 40591-V08H (Sino-biologicals) coating 1 microgr/ml Goat Anti-human IgA (alpha chain specific)-HRP (SIGMA)--Cat: A0295-1ML dilution 1/10000 Purified form of human chimeric IgA anti-RBD of SARS-COV-2 clone IB3C4 (B Cell Design): different concentrations (standard)

Flow cytometry:

Marker	Species/targeting	Fluorochrome	Clone	Supplier	Cat number	Dilution
CD28	mouse anti-human	purified	CD28.2	BioLegend	302933	1:100
CD49d	mouse anti-human	purified	9F10	BioLegend	304339	1:100
CD3	mouse anti-human	BV786	UCHT1	BD Biosciences	565491	1:100
CD4	mouse anti-human	BUV496	SK3	BD Biosciences	612937	1:50
CD8	mouse anti-human	PECy7	RPA-T8	BioLegend	301012	1:100
CD19	mouse anti-human	APC Cy7	HIB19	BioLegend	302218	1:100
CD14	mouse anti-human	APC Cy7	M5E2	BioLegend	301820	1:100
CD56	mouse anti-human	APC Cy7	5.1H11	BioLegend	362512	1:100
IFNγ	mouse anti-human	PE	B27	BD Biosciences	554701	1:50
Fixable Viability Dye		eF780		eBioscience	65-0865-14	1:500
CD3	mouse anti-human	APCFire810	SK7	Biolegend	344858	1:100
CD11c	mouse anti-human	BV785	3.9	Biolegend	301644	1:50
CD19	mouse anti-human	PE Vio770	LT-19	Miltenyi	130-113-170	1:100
CD20	mouse anti-human	BV421	2H7	BD Biosciences	562873	1:100
CD21	mouse anti-human	BUV496	B-ly4	BD Biosciences	750614	1:50
CD27	mouse anti-human	PercP-Vio700	M-T271	Miltenyi	130-113-632	1:100
CD38	mouse anti-human	Viobright FITC	REA572	Miltenyi	130-113-433	1:50
IgD	mouse anti-human	BV605	IA6-2	Biolegend	348232	1:50
IgM	mouse anti-human	PE-CF594	G20-127	BD Biosciences	562539	1:50
IgG	mouse anti-human	BV480	G18-145	BD Biosciences	746341	1:50

Biotinylated recombinant RBD domain of SARS Cov 2 RBD was purchased from Miltenyi Biotech (# 130-127-457) and tetramerized either with streptavidin-PE (BD Biosciences, # 554061) or with streptavidin-APC (Biolegend, # 105243).

Validation

All antibodies used in this study are commercially available, and all have been validated and quality checked by the manufacturers and used in other published works (For references, refer to the supplier's websites :
https://www.bdbiosciences.com/en-eu
https://www.biolegend.com/
https://www.thermofisher.com/fr/fr/home/life-science/antibodies/ebioscience.html
https://www.miltenyibiotec.com/FR-en/.
We also have personal experience of all these antibodies and based on this experience, we can assess the validity of the antibodies. We titrated these antibodies according to our own staining conditions of human PBMCs (usually 1-2 millions cells/100 microliters, 30 min at 4°C).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Vero E6 cells (ATCC CRL-1586) and HEK293T cells (ATCC CRL-1573)

Authentication

We did not authenticate the cell lines, but such authentication is performed by the supplier (ATCC) as part of the quality control

Mycoplasma contamination

All cell lines were regularly screened and tested negative for mycoplasma using a commercial kit (Lonza MycoAlert kit) # LT07-418

Commonly misidentified lines
(See ICLAC register)

no commonly misidentified cell lines were used in the study

Human research participants

Policy information about studies involving human research participants

Population characteristics

Health care workers for COVID-19 vaccinated with Pfizer BNT162b2 and/or AstraZeneca ChadOx01ncov-19 vaccines were included in a prospective longitudinal cohort study conducted in the Hospices Civils de Lyon (Lyon, France). Demographic characteristics are depicted in Table 2.

Recruitment

For the epidemiological investigation in Figure 1 (N=13121), all subjects (health care workers) who were vaccinated with both regimens were included, without exclusion criteria. For the 60 subjects included in the immunological study, we selected 60 subjects who had not been infected by SARS-CoV-2 before vaccination, without comorbidity, and who gave their consent for the study. Written informed consent was obtained from all participants.

Ethics oversight

The use and analysis of data from the occupational health medical file was authorized after a regulatory declaration to the National Commission for Information Technology and Civil Liberties (CNIL) according to the reference methodology (declaration MR004 n° 20-121 of 30 april 2020).
Covid-ser-vac: ethics approval was obtained from the national review board for biomedical research in April 2020 (Comité de Protection des Personnes Sud Méditerranée I, Marseille, France; ID RCB 2020-A00932-37)

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT04341142
Study protocol	We amended the first version of protocol (https://bmjopen.bmj.com/content/10/11/e041268) with the aim to include vaccinated HCWs _ favorable amendment obtained 15th January 2021 from the comité de Protection des Personnes Sud Méditerranée I, Marseille, France; ID RCB 2020-A00932-37).
Data collection	For analyses of infections (Figure1), Health Care Workers at the Lyon University hospital (HCL) who received each vaccination regimen were monitored for infections starting in January 2021 in both groups. Data in Figure 1 show the infections that occurred after the 14-days postboost period, up to the end of the recording (08/15/2021) For immunological analyses (Figures 2-3), clinical data were collected using the Clinsight software, during January-April 2021. . Blood samples were processed and stored at the Centre de Ressource Biologique Neurobiotec, 69500 Bron. Serological and immunological analyses were performed at the Lyon-Sud hospital or at the Centre International de recherche en infectiologie (CIRI) in Lyon.
Outcomes	For figure 1 (analyses of infections) the primary outcome was the infection status ie not infected vs infected. This was assessed by performing RT-PCR for SARS-CoV-2 upon contact with confirmed cases or upon symptoms onset. For figures 2 and 3, (covid-ser study), the primary outcome was the positivity of the SARS-Cov-2 serological test at different time points, and the secondary outcome was the serum level of IgM and IgG titers and the serum neutralization capacity.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	For T cell stimulation and staining: Cryopreserved PBMCs were thawed and rested overnight in RPMI 1640 medium supplemented with 10% FBS in 5% CO2 at 37°C. On the second day, cytokine-producing T cells were identified by intracellular cytokine staining (ICS): Briefly, the PBMCs were stimulated with individual peptides for 1 h in the presence of 1 µg/mL monoclonal antibodies against human CD28 (Biolegend) and CD49d (Biolegend) then for an additional 5h with GolgiPlug (brefeldin A, BD Biosciences), GolgiStop (monensin, BD Biosciences). Then a standard antibody staining was carried out: dead cells were first labelled with LIVE/ DEAD™ Fixable eF780 dye (ThermoFisher) and then followed by surface antibody staining. Subsequently, Cytofix/Cytoperm kit (BD Biosciences) was used for permeabillizing the cells before staining the cells with antibodies against molecules expressed intracellularly. For SARS-CoV-2 RBD-specific B cells analysis : PBMC staining were clearly described in the methods section of the manuscript. Briefly, cryopreserved PBMCs were centrifuged and resuspended in PEB Buffer (PBS + 0.5% BSA and 2 mM EDTA) and incubated with Fc receptor block (Miltenyi 130-059) for 15 min at 4°C. Next, cells were washed in PEB and stained for 30 min in brilliant stain buffer at 4°C in the dark using surface antibodies with both the PE- and APC-conjugated recombinant RBD tetramers. Then, cells were washed in PEB, and resuspended in a PEB dilution of the fixable viability dye eFluor 780 (ebiosciences 65-0865-18). They were next washed and fixed in 4% paraformaldehyde (PFA) for 20 min at 4°C in the dark before a final wash and resuspension for flow cytometry analysis.
Instrument	BD LSRFortessa 5L (T cell) or Cytek Aurora spectral flow cytometer 5L (B cell)
Software	Flow cytometry data were collected with BD FACSDIVA v8.01 or SpectroFlo V2.2.0 (Cytek) and analyzed with FlowJo software version 10.7.1 FlowJo LLC)
Cell population abundance	Bulk PBMC were used. No cell sorting was performed.
Gating strategy	Peptide-specific T cell subsets were identified via the following gating strategy: Viable lymphocytes were addressed by successive gating in SSC-A/FSC-A plot followed by excluding DUMP positive cells (dead cells, CD14, CD19, CD56) in a FSC-A/ DUMP plot. Then, singlets were gated in a FSC-A/FSC-H plot and CD3+ T cells were gated in CD3/FSC-A plot. From CD3+ T cells, CD4+ and CD8+ T cells were gated in CD4/CD8 plots. Next, peptide-specific CD4 T cells were gated by plotting CD4/IFNg and peptide-specific CD8 T cells were gated by plotting CD8/IFNg. Gating strategy for RBD-specific B cell analysis is provided in the supplementary information file and cell population are defined. Briefly, successive gating were applied for single cells and viable cells. Then, B cells were gated as CD19+/CD3- cells.

Within the B cell gate, memory B cells (mBCs) were defined as non-naïve/non transitional B cells on the same biparameter CD27/IgD plot. Then, PE- (RBD#1) and APC- conjugated (RBD #2) SARSCoV2 RBD probes in B cell gate and memory B cell gate were gated in RBD#1/ RBD#2 plot. Unswitched (IgD+CD27+), and switched (csM, IgD-CD27+) RBD-specific memory B cells were defined. Next, IgG+ and IgG-/IgM switched RBD-binding memory B cells were defined based on the IgM/IgG plot.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.