Immunogenicity and efficacy of heterologous ChAdOx1–BNT162b2 vaccination

The ChAdOx1–S-nCoV-19 and BNT162b2 vaccines (hereafter referred to as ChAd and BNT, respectively) have been shown to confer strong protection against SARS-CoV-2 infection and to induce robust immune responses directed against the spike protein of SARS-CoV-2 when administered in a homologous setting. However, the effectiveness of a ChAd–BNT combination as a prime–boost regimen has not been formally tested in vaccine trials. To compare the risk of SARS-CoV-2 infection following heterologous ChAd–BNT or homologous BNT–BNT vaccination schedules, we extracted data from the occupational medicine database of the University Hospital of Lyon (Hospices Civils de Lyon), France. Specifically, we analysed the number of individuals who received each vaccination regimen (which started in January 2021 in both groups) and the number of SARS-CoV-2 infections (documented by a positive PCR with reverse transcription (RT–PCR) result) that occurred at least 2 weeks after the booster dose in each group (Table 1). Ten infections were identified out of 2,512 individuals (0.40%) in the heterologous vaccination group compared with 81 infections out of 10,609 individuals (0.76%) in the homologous vaccination group (Fig. 1). As the age of the individual and the vaccination regimen are known covariates, we used a multiple logistic regression model to model the probability of being infected. Our predictors were age (set as a continuous variable, considered as a possible confounder) and the vaccination regimen. Results showed that age is not associated with infection status ($P = 0.4514$). In particular, patients aged 60 years or above (715 out of 13,121) were not more infected (3 out of 715; 0.42%) than patients aged 60 years or below (88 out of 12,406 (0.71%); two-sided Fisher’s exact test, $P = 0.4890$). Following severe adverse reactions to the AstraZeneca ChAdOx1-S-nCoV-19 vaccine, European health authorities recommended that patients under the age of 55 years who received one dose of ChAdOx1-S-nCoV-19 receive a second dose of the Pfizer BNT162b2 vaccine as a booster. However, the effectiveness and the immunogenicity of this vaccination regimen have not been formally tested. Here we show that the heterologous ChAdOx1-S-nCoV-19 and BNT162b2 combination confers better protection against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection than the homologous BNT162b2 and BNT162b2 combination in a real-world observational study of healthcare workers ($n = 13,121$). To understand the underlying mechanism, we conducted a longitudinal survey of the anti-spike immunity conferred by each vaccine combination. Both combinations induced strong anti-spike antibody responses, but sera from heterologously vaccinated individuals displayed a stronger neutralizing activity regardless of the SARS-CoV-2 variant. This enhanced neutralizing potential correlated with increased frequencies of switched and activated memory B cells that recognize the SARS-CoV-2 receptor binding domain. The ChAdOx1-S-nCoV-19 vaccine induced a weaker IgG response but a stronger T cell response than the BNT162b2 vaccine after the priming dose, which could explain the complementarity of both vaccines when used in combination. The heterologous vaccination regimen could therefore be particularly suitable for immunocompromised individuals.
Vaccinated participants

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Antibodies and virus neutralization

To understand the immunological basis of this difference, we assem-
bled a cohort of health care workers (HCWs) without comorbidity (Covid-Ser-Vac). Half of this HCW cohort received the homologous BNT–BNT combination within the recommended 4-week interval between the two doses, whereas the second half received the BNT booster after receiving the first ChAd dose approximately 12 weeks before. For both groups, the mean age was 41 years and 34 years, respectively, and about 70% of participants were female (Table 2). Three blood samples were drawn: before vaccination, before the second dose and 4 weeks after the second dose. Immunological analyses were performed longitudinally (Extended Data Fig. 1), and a linear regression model was used to compare immune parameters between groups to adjust for age differences.

As previously shown6,7, the BNT vaccine induced a stronger level of SARS-CoV-2 receptor binding domain (RBD) or spike S1-specific IgG than the ChAd vaccine after the first dose. However, these IgG titres were no longer statistically different between groups after the second dose (Fig. 2a, b). Moreover, the positivity rate was 100% for both heterologous and homologous vaccinations. Serum IgA levels measured after the booster vaccination tended to be greater in the homologous than in the heterologous setting (median (interquartile range (IQR)) of 46.7 ng m⁻¹ (36.30–78.70 ng m⁻¹)(Eq) versus 37.4 ng ml⁻¹ (25.40–59.80 ng ml⁻¹)(Eq), respectively; equivalence was to the standard used (Methods)) (Fig. 2c).

We then measured the ability of serum samples to neutralize SARS-CoV-2 spike-pseudotyped viral particles6. None of the sera displayed a detectable neutralizing activity before vaccination or after the first dose. However, most of the sera displayed a neutralizing activity after the second dose, and those from the ChAd–BNT vaccinated individuals displayed a higher neutralizing efficacy than those from the BNT–BNT vaccinated individuals (median (IQR) of 62% (34–93%) versus 99% (89–100%) of neutralization, respectively; \( P < 0.001 \) (Fig. 2d).

To validate these results, we tested the ability of the sera to prevent Vero E6 cell infection by SARS-CoV-2 isolates belonging to various clades, including 19A (B38 lineage), Alpha (B1.1.7 lineage), Beta (B.1.351 lineage), Gamma (P1) and Delta (B.1.617.2 lineage)6. Each SARS-CoV-2 isolate was

**Table 1 | Infection rate in vaccinated HCWs at the Lyon University Hospital**

| Naive HCWs | BNT–BNT | ChAd–BNT |
|------------|---------|----------|
| Numbers of participants | 10,609 | 2,512 |
| Median age (minimum–maximum) | 42 (19–76) years⁴ | 33 (19–64) years⁴ |
| Numbers of infected participants (%) | 81 (0.76)⁵ | 10 (0.4)⁵ |
| Median age (minimum–maximum) | 41 (22–65) years | 32 (22–49) years |

⁴ Two-sided Student’s \( t \)-test, \( P = 5 \times 10^{-4} \)

**Table 2 | Clinical characteristics of patients in the Covid-Ser-Vac study**

| | Heterologous vaccine | Homologous vaccine | \( P \) value | Adjusted \( P \) value |
|----------------|---------------------|-------------------|--------------|------------------------|
| \( n = 29 \) | \( n = 31 \) | \( n = 29 \) | \( n = 31 \) |
| Male sex (%(n)) | 9 (31.03) | 8 (25.80) | 0.7742 | 0.7742 |
| Age in years (median (IQR)) | 34 (27–40) | 41 (33–52) | 0.0016 | 0.0056 |
| Body mass index (n) | 28 | 31 |
| Median (IQR) | 22.25 (20.7–24.3) | 23.12 (21.45–25.83) | 0.2279 | 0.3988 |
| Currently smoker (%(n)) | 5 (17.24) | 8 (25.8) | 0.5358 | 0.6251 |
| Alcohol consumption¹ (%(n)) | 7 (24.13) | 5 (16.12) | 0.5269 | 0.6251 |

¹ Logitstic regression model, \( P = 0.6384 \)

**Fig. 1 | Incidence of SARS-CoV-2 infection after different vaccination regimens.** Histograms show the infection rate (as documented by a positive SARS-CoV-2 RT–PCR result) among groups of HCWs who were vaccinated with the homologous BNT–BNT combination (\( n = 81 \) out of 10,609) within the recommended 4-week timeframe between the two doses or with the BNT boost after receiving the first ChAd dose (\( n = 10 \) out of 2,512) approximately 12 weeks before, as recorded by the service of occupational medicine, Hospices Civils de Lyon. Data show the infection rate that occurred for age differences.

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Fig. 2 | Heterologous vaccination induces higher neutralizing antibody titres. a–c, Sera from ChAd–BNT (n = 29) or BNT–BNT (n = 31) individuals were assayed for S1-specific IgG (a), RBD-specific IgG (b) or S1-specific IgA (c) levels using commercial or custom-made ELISA tests at different times during the vaccination process as indicated (a, b) or 4 weeks after full vaccination (c). In a–c, concentrations are expressed in binding antibody units per ml (BAU ml$^{-1}$) or ng ml$^{-1}$ Eq of immunoglobulin as indicated. Each serum sample was evaluated as a single measurement (a, b) or in triplicate (c). Dotted lines in a and b indicate positive detection according to the manufacturer. d–f, Sera from ChAd–BNT (n = 29) or BNT–BNT (n = 33) vaccinated individuals were assayed in triplicate for their capacity to neutralize the entry of virus-like particles pseudotyped with the Wuhan strain SARS-CoV-2 envelope (d) or in duplicate for their capacity to neutralize infection of Vero E6 cells by different SARS-CoV-2 strains (e, f), as indicated. Data show the per cent of neutralization relative to a positive control (d) or the 50% plaque reduction neutralization test (PRNT50) (e), and are expressed as dot plots, with one dot corresponding to one individual. The limit of detection is shown as a dotted line in e. FC, fold change in the mean of the indicated groups. In all panels, box-and-whiskers plots are shown (see Methods for details), and the median is represented by the magenta middle line. A linear regression model was used to compare values between groups, and this model was corrected for age. Exact P values are shown for the indicated comparisons when significant or nearly significant. f, Comparison of serum neutralizing activity against the reference lineage 19A and against the variants of concern for each group. P values are shown and calculated using the linear regression model described in e.

 sequenced and confirmed to contain the characteristic mutations of its viral clade. The ChAd–BNT vaccinated individuals exhibited 2.3-fold to 3.6-fold higher serum neutralizing antibody titres against the different variants than BNT–BNT vaccinated individuals (Fig. 2e). Moreover, in the latter group, the neutralizing responses observed against the Beta, Gamma and Delta variants were significantly reduced compared with that against the 19A clade. By contrast, the neutralizing activity detected in sera from ChAd–BNT vaccinees was similarly high against all strains except the Beta clade (Fig. 2e, f). These data demonstrate that the neutralizing potential of antibodies generated by heterologous vaccination is less affected by spike mutations appearing in variants of concern than that of antibodies induced by homologous vaccination.

Vaccine-induced memory cells

We then studied the memory B cell (mBC) compartments using fluorescently labelled tetrameric RBDs to track RBD-specific mBCs and to analyse their phenotype (the gating analysis is presented in Extended Data Fig. 2a). As shown in Fig. 3a and Extended Data Fig. 2b, which correspond to concatenated flow cytometry plots, the frequency of RBD-binding mBCs was comparable for both vaccination groups after the prime dose but was on average twofold higher for the ChAd–BNT vaccination schedule after the booster dose. Moreover, switched (IgD ‘CD27$^+$’) mBCs accounted for about 67% and 47% of the RBD-specific mBC pool in the ChAd–BNT group and the BNT–BNT group, respectively (Fig. 3b, c, Extended Data Fig. 2c). The expression pattern of IgM and IgG isotypes was next used to compare the immunoglobulin isotype distribution among the switched mBCs. The results showed that the homologous vaccination schedule promoted more IgG-switched mBCs (62% versus 48%) than the heterologous vaccination schedule (Fig. 3d, Extended Data Fig. 2d). Finally, to address the activation status of mBCs, we analysed their expression of CD21 and CD11c. It is generally considered that the concurrent loss of CD21 and the upregulation of CD11c are phenotypic features associated with B cell activation independent of the developmental stage 10. Resting mBCs were therefore defined as CD21$^+$CD11c$^-$ cells, whereas CD21$^-$CD11c$^+$, CD21$^+$CD11c$^+$ and CD21$^+$CD11c$^-$ cells were globally considered as activated mBCs. The frequencies of activated RBD-specific mBCs were significantly enhanced in the ChAd–BNT group compared with the BNT–BNT group (Fig. 3e, Extended Data Fig. 2e). In particular, the proportions of the CD21$^+$CD11c$^-$ subset were almost three times higher in the ChAd–BNT group. Altogether, our results indicate that the ChAd–BNT combination induces significantly more RBD-specific B cells, increases the frequencies of post-switch mBCs and induces a more active mBC generation process.
We then longitudinally monitored the T cell response of vaccinees against RBD peptides using a whole-blood interferon-γ (IFNγ) release assay. The T cell response was higher after ChAd immunization than after BNT priming and was similarly increased by the BNT booster dose in both groups (median of 0.33 U/ml versus 0.43 U/ml) (Fig. 3f). To confirm and consolidate these results, we measured the spike-specific CD4 and CD8 T cell response at the single-cell level by flow cytometry after stimulating peripheral blood mononuclear cells (PBMCs) from vaccinees with a pool of commercial peptides spanning the entire spike protein and then staining for intracellular IFNγ (the gating strategy is presented in Extended Data Fig. 3). Both vaccination regimens were able to induce a progressive increase in the frequency of S-reactive CD4 and CD8 T cells from the pre-vaccine to the post-booster phase (Fig. 3g, h). However, the heterologous combination resulted in a stronger CD4 T cell response, both after priming and boosting, and in an increasing trend in the CD8 T cell response after the booster dose.

Finally, to understand interrelations between immune parameters, we performed a matrix analysis (Extended Data Fig. 4). This confirmed that the neutralizing antibody activity correlated with the titres of spike-specific IgG, regardless of the variant analysed, and not correlated with IgA levels. Moreover, the neutralization activity correlated with the percentage of switched RBD+ mBCs and with the percentage of activated RBD+ mBCs. This provides further support that better neutralizing antibodies are produced as a result of sustained B cell activation.

**Discussion**

Heterologous prime–boost vaccinations have been reported to be more immunogenic than homologous ones in experimental settings. This has also been proven for some human vaccines. Moreover, studies using mice have demonstrated the strong immunogenicity of the ChAd–BNT combination. Here we report that heterologous ChAd–BNT vaccination
concluded that for short intervals, the humoral responses induced by both regimens were comparable. However, a longer interval (that is, 12 weeks) between prime and boost could be needed to allow the synergy between heterologous vaccines.

Together, we present a real-world observational study of HCWs showing that the heterologous ChAd–BNT vaccination regimen confers stronger protective immunity than the homologous BNT–BNT prime–boost schedule. As hidden confounding factors might be present in our study (for example, different levels of exposure to the virus), confirmatory studies and a longer follow-up of vaccinated participants are warranted. However, our data suggest that the heterologous combination could be particularly suitable for immunocompromised individuals.

Online content
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Covid-Ser study group

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Methods

Ethical statement and cohort description
The use and analysis of data from the occupational health medical file were authorized after a regulatory declaration to the National Commission for Information Technology and Civil Liberties according to the reference methodology (declaration MR004 number 20-121 of 30 April 2020). The declaration of SARS-CoV-2 infection is compulsory for all staff to obtain daily allowances without loss of salary during the imposed quarantine.

For the Covid-Ser-Vac study, clinical data were recorded by a trained clinical research associate using Clinisight software (v.Csonline 7.5.720.1). Blood samples were processed and stored at the Centre de Ressource Biologique Neurobiotest, 69500 Bron. Sixty naïve HCWs for COVID-19 and vaccinated with BNT and/or ChAd vaccines were included in a prospective longitudinal cohort study conducted at the Hospices Civils de Lyon. Blood sampling was performed before vaccination, before the second dose of vaccine and 4 weeks after the end of vaccination schedule. The absence of previous SARS-CoV-2 infection was confirmed using the Wantai SARS-CoV-2 Ab total assay in the pre-vaccine sample. Demographic characteristics and delays in infection was calculated using the calibration curve. The limit of detection of the assay was 0.1 ng ml⁻¹ Eq.

Sera were immediately stored at −80 °C after blood sampling. SI-specific IgG and RBD-specific IgG were measured using Siemens Atellica IMSARS-CoV-2 IgG (sCOVG) and bioMérieux Vidas SARS-CoV-2 IgG diagnosis kits, respectively, according to the manufacturers’ recommendations. For standardization of these assays to the first World Health Organization international standard, the concentrations were transformed into binding antibody units per ml (BAU ml⁻¹) using the conversion factors provided by the manufacturers.

Measurement of IgG titres
ELISAs to evaluate IgA binding to the SARS-CoV-2 spike protein were performed as previously described. High-binding 96-half-well plates (2310M, Nunc) were coated with 100 µl per well of a spike protein solution (1 µg ml⁻¹; 40591-V08H spike SI-RBD, Sino Biologicals) in PBS overnight at 4 °C. Plates were washed with washing buffer containing 1x PBS with 0.05% Tween 20 (Sigma-Aldrich) and incubated with 170 µl of blocking buffer per well containing 1x PBS with 3% fat milk powder and 0.05% Tween 20 (Sigma-Aldrich) for 1 h at room temperature. Immediately after blocking, recombinant anti-RBD IgA (B Cell Design, IB3C4 PV) or serum samples diluted in PBS were added and incubated for 1 h at 37 °C. Plasma samples were assayed at a 1:100 starting dilution and 7 additional 3-fold serial dilutions. Recombinant human anti-RBD IgA was used to perform a calibration curve starting at 1.5 µg ml⁻¹. Plates were washed and then incubated with anti-human IgA (A0295, Sigma-Aldrich) secondary antibody conjugated to horseradish peroxidase in blocking buffer at 1:10,000. Plates were developed by the addition of the horseradish peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB; 34021, Thermo Fisher Scientific) for 10 min, and the developing reaction was stopped by adding 50 µl of 1 M HCl. Optical density units were measured at 450 nm in a microplate reader (Tecan). For serum samples, a positive control (serum pool from critically ill patients with COVID-19, diluted 200-fold in PBS) and a negative control (pool of historical serum samples) were added in duplicate to each run. After deduction of the background, a relative content in IgA equivalent (ng ml⁻¹ Eq) was calculated using the calibration curve. The limit of detection of the assay was 0.1 ng ml⁻¹ Eq.

Live-virus neutralization experiments
A plaque reduction neutralization test (PRNT) was used for the detection and titration of neutralizing antibodies. A tenfold dilution of each serum specimen in culture medium was first heated for 30 min at 56 °C to avoid complement-linked reduction of viral activity. Serial twofold dilutions (tested in duplicate) of the serum specimens in culture medium were mixed in equal volume with the live SARS-CoV-2 virus. After gentle shaking and a contact of 30 min at room temperature in plastic microplates, 150 µl of the mix was transferred into 96-well microplates covered with Vero E6 cells (American Type Culture Collection (ATCC), CRL-1586, not authenticated but regularly tested for mycoplasma contamination). The plates were incubated at 37 °C in a 5% CO₂ atmosphere. Infection efficiency was evaluated by microscopy 5 days later when the cytopathic effect of the virus control reached 100–500 TCID₅₀ (median culture infectious dose) per 150 µl. Neutralization was recorded if more than 50% of the cells present in the well were preserved. The neutralizing titre was expressed as the inverse of the higher serum dilution that exhibited neutralizing activity: a threshold of 20 was used (PRNTₜᵯₑᵣₜ ≥ 20). All experiments were performed in a biosafety level 3 laboratory. The different viral strains that were used were sequenced and deposited at GISAID (https://www.gisaid.org/) (accession numbers EPI_ISL_1707038 19A (B.38 lineage); EPI_ISL_1707039 Alpha (B.1.1.7 lineage); EPI_ISL_768828 Beta (B.1.351 lineage); EPI_ISL_1339892 Gamma (P.1 lineage); and EPI_ISL_1904989 Delta (B.1.617.2 lineage)).

Monitoring of T cell responses using whole-blood IFNγ release assay
Fresh blood collected in heparinized tubes was stimulated for 22 h at 37 °C under 5% CO₂ with SARS-CoV-2 peptide pools (derived from the prototype Wuhan strain NC_045512.2) targeting RBD (46 peptides, [C] = 8 µg ml⁻¹) (bioMérieux) and MHC class I peptides pools against SARS-CoV-2 spike protein (PepTivator, Miltenyi Biotec) at a final concentration of 1 µg ml⁻¹ for 1 h in the presence of 1 µg ml⁻¹ mononclonal antibodies CD28 and CD49d, and then for an additional 5 h with GolgiPlug and GolgiStop (BD Biosciences). Dead cells were labelled using Fixable Viability eFluor 780 dye (Thermo Fisher Scientific). Surface markers were stained using BV786-conjugated anti-CD3 (BD Biosciences, 612937; 1:50), PE-Cy7-conjugated anti-CD8 (BioLegend, 301820; 1:100), APC-Cy7-conjugated anti-CD14 (BioLegend, 301012; 1:100), APC-Cy7-conjugated anti-CD19 (BioLegend, 302218; 1:100) and APC-Cy7- conjugated anti-CD56 (BioLegend). The measuring range was 0.08–8 IU ml⁻¹. The IFNγ response was defined as detectable when the IFNγ concentration of the test minus IFNγ concentration of the negative control was above threshold or when the IFNγ concentration of the test minus IFNγ concentration of the negative control was below threshold.

Monitoring of T cell responses by flow cytometry
Overnight-rested PBMCs were stimulated with SARS-CoV-2 overlapping peptide pools against SARS-CoV-2 spike protein (PepTivar, Miltenyi Biotec) at a final concentration of 1 µg ml⁻¹ for 1 h in the presence of 1 µg ml⁻¹ monoclonal antibodies CD28 and CD49d, and then for an additional 5 h with GolgiPlug and GolgiStop (BD Biosciences). Dead cells were labelled using Fixable Viability eFluor 780 dye (Thermo Fisher Scientific). Surface markers were stained using BV786-conjugated anti-CD3 (BD Biosciences, 565491; diluted 1:100), BU4V86-conjugated anti-CD4 (BD Biosciences, 612937; 1:50), PE-Cy7-conjugated anti-CD8 (BioLegend, 301012; 1:100), APC-Cy7-conjugated anti-CD4 (BioLegend, 301820; 1:100), APC-Cy7-conjugated anti-CD56 (BioLegend, 362512; 1:100) and APC-Cy7- conjugated anti-CD19 (BioLegend, 302218; 1:100). Cells were then washed, fixed with Cytofix/Cytoperm (BD Biosciences) and stained with PE-conjugated anti-IFNγ (BioLegend). Negative controls without peptide stimulation were run for each sample. All results were acquired on a BD LSRFortessa (BD Biosciences) flow cytometer using the BD FACSDiva v.8.01 software and analysed using FlowJo v10.6.1 software.

SARS-CoV-2 pseudoparticle preparation and neutralization
SARS-CoV-2 spike-pseudotyped murine leukaemia virus retrovirus particles were produced as described for SARS-CoV10. In brief, HEK293T particles were produced as described for SARS-CoV10. In brief, HEK293T
cells (ATCC, CRL-1573, not authenticated but regularly tested for mycoplasma contamination) were transferred with constructs expressing murine leukaemia virus Gag-Pol, the green fluorescent protein (GFP) reporter and the SARS-CoV-2 spike protein (a gift from D. Lavillette (CAS Key Laboratory of Molecular Virology & Immunology, Institut Pasteur of Shanghai Chinese Academy of Sciences, Pasteurien College, Soochow University, Jiangsu, China)). Control particles pseudotyped with the unrelated RD14 virus surface glycoprotein (from a cat endogenous virus) were generated as previously described. For neutralization assays, a sample of approximately 1 × 10⁶ pseudoparticles was incubated with a 100-fold dilution of sera or control antibodies for 1 h at 37°C, spun incubated for 2 h at 2,500g before infection of Vero E6 cells. After 72 h of infection, the percentage of GFP-positive cells was determined by flow cytometry. As a control, the same procedure was performed using RD14 pseudoparticles. Anti-spike SARS-CoV-2 RBD (Sino Biological, 40150-V08B2) and anti-gp70 RD14 (ViroMed Biosafety Labs) antibodies were used with a 100-fold dilution as positive and negative control, respectively.

Generation of fluorescent SARS-CoV-2 RBD tetramers
Biotinylated recombinant domain of SARS-CoV-2 RBD was purchased from Miltenyi Biotech (130-127-457) and tetramerized with either streptavidin-PE (Becton Dickinson (BD)) or with streptavidin-APC (BioLegend, 103243).

Flow cytometry analysis of SARS-CoV-2 RBD-specific B cells
Cryopreserved PBMCs were centrifuged and suspended in PEB buffer (PBS with 0.5% BSA and 2 mM EDTA) and incubated with Fc receptor block (Miltenyi, 130-059-901) for 15 min at 4°C (dilution 1:10). Next, cells were washed in PEB and stained for 30 min in brilliant stain buffer at 4°C in the dark using the following antibodies together with both the PE- and APC-conjugated recombinant RBD tetramers: anti-CD3-APC Fire 610 (BioLegend, 344858; diluted 1:100); anti-CD11c-BV785 (BioLegend, 301644; 1:50); anti-CD19-PE Vio770 (Miltenyi, 130-113-70; 1:100); anti-CD20-BV421 (BD, 562873; 1:100); anti-CD21-BUV496 (BD, 750614; 1:50); anti-CD27-PercP-Vio700 (Miltenyi, 130-113-632; 1:100); anti-CD38-VioBright FITC (Miltenyi, 130-113-433: 1:50); anti-IgM-PE-CF594 (BD, 562359; 1:50); anti-IgG-BV605 (BioLegend, 348232; 1:50); and anti-IgD-BV480 (BD, 746341; 1:50). Cells were washed in PEB and resuspended in a PEB dilution (1:500) of the fixable viability dye eFluor 780 (eBiosciences, 65-0865-18). They were next washed and fixed with 4% parformaldehyde for 20 min at 4°C in the dark before a final wash and resuspension for analysis. Cells were then acquired on a Cytek Aurora spectral flow cytometer equipped with five lasers operating at 355, 405, 488, 561 and 640 nm using the SpectroFlo V.2.2.0 (Cytek) software. Data were analysed using FlowJo 10.6.1 software (BD).

Statistical analysis for immunological and virological analyses.
ChAd–BNT and BNT–BNT populations showed a significant difference in the average ages. To test for the consequence of the vaccination regimen on the different immune parameters, we fitted a multiple linear regression model, which allows for the correction of age (as a possible confounding factor) by including age and vaccination regimen simultaneously as predictors in the right explanatory side of the lm function in R (one model per immune parameter). Results of statistical significance displayed in Figs. 2 and 3 are from these adjusted models, for which age showed no effect. For box and whiskers plots, the upper and lower bounds of the box are the 75th and the 25th percentile (the third Q3 and first Q1 quartile, respectively). The IQR = Q3 – Q1; an observation was considered an outlier if it was above Q3 + 1.5 × IQR or below Q1 – 1.5 × IQR. Upper and lower whiskers represent the maximum and the minimum values, respectively, without taking into account the outliers.

Statistical modelling for epidemiological data.
To model the probability of being infected knowing the subject age and the used vaccination regimen, we used the generalized linear model (glm) function in R to perform a multiple logistic regression model with the logit link function. The outcome variable was the infection status, whereby infected = 1 and not infected = 0. Our predictors were the age, as a continuous variable (considered as a possible confounder), and the vaccination regimen, whereby ChAd–BNT = 0 and BNT–BNT = 1. On the basis of the model coefficients for the vaccination regimen (B0 = −3.306 and B1 = 0.712), we also assessed the relative risk (RR) as follows: RR = (1 + exp(−B0))/(1 + exp(−B0 − B1)).
Extended Data Fig. 1 | Study design. Sixty HCWs naïve for COVID-19 and vaccinated with the Pfizer ChAd/BNT or the BNT/BNT combination were included in a prospective longitudinal cohort study. Blood sampling was performed as described, before vaccination, before the second dose of vaccine and 4 weeks after the end of vaccination schedule. Different immunological analyses were performed on the blood samples, including serological investigations (Spike-specific IgA and IgG), serum neutralization assays (both plaque reduction neutralization test (PRNT) and pseudoneutralization), analysis of Spike-specific T cells and RBD-specific memory B cells, at various time points indicated in the text. Parts of the figure were drawn by using pictures from Servier Medical Art (http://smart.servier.com/), licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).
Extended Data Fig. 2 | Analysis of RBD-specific memory B cells. (A) Gating strategy. From left to right, the first three pseudo-color plots show the successive gates applied for single cells and viable cells. B cells were gated as CD19+/CD3- cells (4th plot). Within the B cell gate, memory B cells (mBCs) were defined as non-naïve B cells on the same biparameter plot (5th plot). (B-E) Concatenated phenotypic profiles of RBD-specific mBCs for all individuals in the ChAd/BNT and BNT/BNT groups at three time points (before vaccination, before the 2nd dose, after the second dose). For each group, the phenotypic profiles correspond to the concatenation of the FACS analysis data collected from 30 individual vaccinees. (B) Visualization of RBD-binding B cells in the mBC gate. (C) Visualization of the proportion of switched (IgD-) and unswitched (IgD+) B cells among the RBD-specific mBC pool. (D) Visualization of the pattern of Ig isotype expression by RBD-specific mBCs. (E) Visualization of the distribution of RBD-binding B cells within the resting (CD21-CD11c-) and “activated” (CD21-CD11c+/CD21-CD11c-/CD21+CD11c+) mBC compartments. The mean frequencies of RBD-specific mBCs falling into each quadrant are indicated in red.
Extended Data Fig. 3 | Analysis of SARS-CoV-2-specific T cells. PBMCs were stimulated as detailed in the methods and stained for CD3, CD4, CD8, CD14, CD56 and CD19 expression and with a dead cell marker. Cells were gated from left to right and top to bottom. The Viab/dump channel includes antibodies against CD14, CD19, CD56 and a viability marker. The frequency of CD4 or CD8 T cells positive for intracellular IFNg was measured as shown in the central and right bottom panels.
Extended Data Fig. 4 | Analysis of inter-relationships between immune parameters. Analysis of the inter-relationships of the immunological measurements shown in Supplementary Table 3. A matrix file with all immunological measurements for all vaccinated individuals showing the Spearman correlations and associated p-values. The size and the color of the circles correspond to the correlation, as indicated, and the stars indicate the significance of the correlation.
Reporting Summary

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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 concomitance 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 excluded 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 immunological 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

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    |
| CD4    | 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      | S1H11 | BioLegend     | 362512     | 1:100    |
| IFNγ   | mouse anti-human  | PE           | B27   | BD Biosciences| 554701     | 1:50     |

Flexible 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.miltenyibiotech.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 (momensin, 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 permeablizing the cells before staining the cells with antibodies against molecules expressed intracellularly.

For SARS-CoV-2 RBD-specific B cells analysis :
PBM 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 resupernanted 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/IgFg and peptide-specific CD8 T cells were gated by plotting CD8/IgFg.

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-naive/non transitional B cells on the same biparameter CD27/IgD plot. Then, PE- (RBD#1) and APC- conjugated (RBD #2) SARS-CoV2 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.