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Immunogenicity and efficacy of heterologous ChadOx1/BNT162b2 vaccination

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33 Following severe adverse reactions in patients vaccinated with the
34 AstraZeneca ChadOx1 (Chad) vaccine, European health authorities have
35 recommended that patients under the age of 55 who received one dose of
36 Chad vaccine receive a second dose of Pfizer BNT162b2 (BNT) vaccine as a
37 booster. However, the effectiveness and the immunogenicity of this
38 vaccination regimen have not been formally tested. Here, we show that the
39 heterologous Chad/BNT combination confers better protection against SARS-
40 CoV-2 infection than the homologous BNT/BNT combination in a population of
41 health care workers. To understand the underlying mechanism, we monitored
42 in a longitudinal way the anti-spike immunity conferred by each vaccinal
43 combination. Both combinations induced strong anti-spike antibody responses
44 after boost in all vaccinated individuals. However, sera from heterologous
45 vaccinated individuals displayed a stronger neutralizing activity, regardless of
46 the SARS-CoV-2 variant analyzed, and this was associated with more switched
47 memory RBD-specific B cells with an activated phenotype and less IgA. The
48 Chad vaccine induced a stronger T cell response than the BNT vaccine after
49 the priming dose, and the reciprocal was true for the IgG response, which
50 could explain the complementarity of both vaccines when used in an
51 heterologous setting. This strongly protective vaccination regimen could be
52 therefore particularly suitable for immunocompromised individuals.

53 Chad and BNT vaccines have been shown to confer strong protection against SARS-
54 CoV-2 infection, and to induce robust immune responses directed against SARS-
55 CoV-2 Spike protein, both at the cellular and humoral level, when administered in a
56 homologous setting¹⁻³. However, the effectiveness and the immunogenicity of a
57 Chad/BNT combination as prime/boost vaccination has not been formally tested in

clinical trials. In order to compare the risk of SARS-CoV-2 infection following heterologous Chad/BNT or homologous BNT/BNT vaccination schedules, we extracted from the occupational medicine database of medical staff at the university hospital in Lyon, France (HCL) the number of individuals who received each vaccination regimen, and the number of infections documented by positive SARS-CoV-2 RT-PCR that occurred at least 2 weeks after the boost in each group in the past 6 months (Table 1). These infections are documented as part of the administrative formalities related to work stoppages. A single infection was identified out of 2612 individuals in the heterologous group against 34 infections out of 10,216 in the homologous group (Figure 1), this difference being statistically significant (0.04% vs 0.32%, Fischer's exact test $p < 0.01$) in favor of better protection by the heterologous combination. Individuals vaccinated with the heterologous combination were on average younger than in the homologous group (31 vs 43 years old), but the mean age of infected individuals in the homologous group (46, range 22-61) was comparable to that of non-infected ones, suggesting that age was not a major risk factor of infection in this vaccinated cohort.

To understand the immunological basis of the difference in protection, we constituted a cohort of healthcare workers (HCWs) without comorbidity (Covid-Ser-Vac) in which half of HCWs received a homologous BNT/BNT combination within the recommended 4-week timeframe between the two doses, while the second half received the BNT boost after receiving the first Chad dose approximately 12 weeks before. The mean age in both groups was 41 and 34 respectively, and about 70% of participants were women in both groups (Table 2). Three blood samples were drawn for immunological analyses, prior vaccination, prior second dose and then 4 weeks

after the second dose. Immunological analyses were performed in a longitudinal manner as depicted in Figure S1.

As previously shown^{4,5}, the BNT mRNA vaccine induced a stronger level of RBD or Spike S1-specific IgG than the adenovirus Chad vaccine after the first dose. However, the titer of anti-Spike IgG was boosted by the second vaccine dose in both homologous and heterologous settings and became not statistically different between both groups after the second dose (Figure 2A-B). Moreover, the positivity rate was 100% for both heterologous and homologous vaccination. Serum IgA levels measured after the booster vaccination were found to be significantly greater in the homologous than in the heterologous setting (Figure 2C).

We then used a previously developed pseudovirus neutralization assay⁶ using SARS-CoV-2 Spike pseudotyped particles to measure the neutralizing activity of serum samples. None of the sera had a detectable neutralizing activity prior vaccination or after the first dose. However, most of them had neutralizing activity after the second dose, and sera from the Chad/BNT vaccinated patients had higher neutralizing efficacy than those from the BNT/BNT vaccinated ones (median IQR: 62 [34-93] vs 99 [89-100]% of neutralization; $p < 0.001$) (Figure 2D). To further validate these results, the ability of the different sera to prevent VeroE6 cell infection by SARS-CoV-2 isolates belonging to various clades was also tested: 19A (B38 lineage), alpha (B.1.1.241 lineage), beta (B.1.1.7 lineage), gamma (B.1.351 lineage) and delta (B.1.617.2 lineage)⁷. Each SARS-CoV-2 isolate used in this study was sequenced and confirmed to harbor the characteristic mutations of its viral lineage. The Chad/BNT vaccinated individuals exhibited 3-fold higher serum neutralizing antibodies titers against the 19 isolate than individuals from the BNT/BNT group. Moreover, in the latter group, the neutralizing response observed against the beta,

gamma and delta variants was significantly reduced compared to that against the 19A lineage. By contrast, the neutralizing activity of the Chad/BNT vaccinated sera was similarly high against all strains except the beta one against which it was somewhat lower (Figure 2E). These data show that neutralizing antibodies secreted after heterologous vaccination are less affected by Spike mutations appearing in Variants Of Concern (VOCs) than those secreted after homologous vaccination.

We then compared the memory B cell (mBC) compartments induced by the two vaccination schedules using fluorescently labelled tetrameric RBDs to track RBD-specific mBCs and analysed their phenotype (gating analysis Figure S2A). As shown in Figure 3A and S2B, the frequency of RBD-binding mBCs was on average 2 fold superior for the Chad/BNT vaccination schedule. Moreover, as illustrated in Figures 3B-C and S2C, switched (IgD-CD27+) mBCs accounted on average for 67% and 47% of the RBD-specific mBC pool in the Chad/BNT and BNT/BNT groups, respectively. Expression of IgM and IgG was next used to compare the Ig isotype distribution among switched mBCs. Data shown in Figures 3D and S2D reveal that the homologous vaccination schedule promoted more G-switched (62% versus 48%) than the heterologous vaccination schedule. Finally, to address the activation status of mBCs generated by both vaccination schedules, we analysed the pattern of CD21 and CD11c expression among the entire RBD⁺ mBC pool. It is generally considered that the concurrent loss of CD21 and upregulation of CD11c are phenotypical features associated with B cell activation, independently of the B cell developmental stage⁸. Resting mBCs were thus defined as CD21⁺CD11c⁻ cells while CD21⁺CD11c⁺, CD21⁻CD11c⁺ and CD21⁻CD11c⁻ B cells represented different stages of activation and were globally considered as “activated” mBCs. As illustrated by Fig. 3E and S2E, the frequencies of activated RBD-specific mBCs were significantly enhanced in the

ChAd/BNT group as compared to the BNT/BNT group. The proportions of CD21⁺ CD11c⁺ population in particular were almost 3 times higher in the former group of vaccinees than in the latter. Altogether, our results indicate that, as compared to the homologous vaccination schedule, the Chad/BNT vaccinal combination : (i) induces significantly more RBD-specific B cells, (ii) increases the frequencies of post-switch mBCs, and (iii) maintains a more active mBC generation process.

We then monitored in a longitudinal manner the T-cell response of vaccinees against RBD peptides using a whole blood IFN γ release assay. The T cell response tended to be higher after Chad immunization than after BNT priming and was similarly increased by the BNT boost in both groups (Figure 3F). To confirm and refine these results, we also measured the Spike-specific CD4 and CD8 T cell response at the single cell level by flow cytometry after stimulating vaccinees' PBMCs with a pool of commercial peptides spanning the entire protein, and staining for intracellular IFN γ . Results in Figure 3G-H show that 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. However, the heterologous combination resulted in a stronger CD4 T cell response, both after priming and boost.

Finally, to understand interrelationships between immune parameters, we realized a matrix analysis (Figure S3) that confirmed that the neutralizing antibody activity was correlated with S1 or RBD-specific IgG, regardless of the variant analyzed, and not correlated with IgA levels. Moreover, the neutralization activity was correlated with the percentage of switched RBD⁺ mBCs and with the percentage of activated RBD⁺ mBCs, further supporting that better neutralizing antibodies are produced as a result of sustained B cell activation.

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158 Heterologous prime/boost vaccinations are often more immunogenic than
159 homologous ones in experimental settings⁹. This has proven to be the case for some
160 human vaccines as well¹⁰. Moreover, studies in mice showed the strong
161 immunogenicity of the Chad/BNT combination¹¹, supporting its use in humans. Here,
162 we report that the heterologous vaccination Chad/BNT confers better protection
163 against infection, which is associated with more switched memory B cells and higher
164 virus neutralizing antibody titers, irrespective of the variant analyzed. This
165 unexpected finding is of particular importance considering the rise of the SARS-CoV-
166 2 delta variant in Europe¹². Two other studies reported better neutralizing potential of
167 sera from Chad/BNT vaccinated individuals compared to BNT/BNT vaccinated ones,
168 but these studies only used surrogate¹³ or pseudovirus¹⁴ neutralization assays, and
169 not as many variants as in our study. Serological studies based on SARS-CoV-2
170 pseudotypes or chimeric viruses are easy to conduct but their ability to predict
171 neutralizing activity against authentic clinical virus isolates is debated¹⁵. Our own
172 study also has some limitations; i) the mean age in both vaccinated groups was
173 slightly different due to vaccine recommendations; ii) the delay between prime and
174 boost was different in the two studied groups, which could also contribute to the
175 differences observed, beyond the vaccine type; iii) we did not have access to
176 individual data for the infection analysis in Figure 1.

177 At face value it may seem paradoxical that the heterologous vaccination
178 procedure induces Abs with a stronger neutralization potential than the homologous
179 vaccination procedure while the RBD and S-specific IgG antibody titers are
180 comparable in both circumstances. The higher neutralization potential of the Abs
181 generated after heterologous vaccination could be due to their better fit and affinity

for the SARS-CoV-2 Spike protein or to the appearance of B cell clones recognizing epitopes of the Spike protein that are more crucially involved in recognition of the ACE2 cellular receptor. Both scenarios would be compatible with induction of a more efficient or long-lasting germinal center reaction allowing an extended cycle of somatic hypermutations. Two lines of evidence speak in favour of this hypothesis. First, this phenomenon has been observed in convalescent patients over time after infection ^{16,17}, in connection with persistence of viral antigens in the intestine, especially if these individuals were vaccinated after having controlled their infection ¹⁸. Second, we show that the memory B cell compartment generated by the Chad/BNT schedule is characterized by higher frequencies of mBCs (on average twofold higher than in the BNT/BNT vaccination cohort) exhibiting features of activation as expressed by loss of CD21 and upregulation of CD11c. These observations concur to suggest that the process of mBC differentiation at 4 weeks post-boost is more active in the heterologous than in the homologous vaccination schedule. Regarding the biological cues that would drive this possibly extended GC reaction, we observed a higher frequency of Spike-specific T cells after priming with the Chad vaccine, as also observed in other studies ^{4,5}. This stronger response could promote the differentiation of antigen-specific B lymphocytes in the germinal centers and the hypermutation of the antibodies induced during the antigen boost by the BNT vaccine. Moreover, stronger T cell responses may also contribute to better protection independently of B cell responses, through direct or indirect antiviral activity¹⁹.

The better neutralization potential of sera from heterologously vaccinated subjects could also be linked to their lower concentration of S1 specific IgA. Indeed, while mucosal dimeric IgA might be important in fighting infection in the early stages, plasma IgA monomers specific to SARS-CoV-2 proteins were demonstrated to be

twofold less potent than IgG equivalents²⁰. Furthermore, IgA persist better than IgG after infection, which coincides with a drop in the neutralizing potential of the serum¹⁸. Following a possible competition between IgA and serum IgG, the decrease in IgA in heterologous conditions may participate in the better neutralization of the virus and its variants. This phenomenon has already been observed in the context of the RV144 vaccination trial against HIV-1. The level of envelope-specific IgA correlated with the risk of infection of vaccinated patients²¹, and in vitro results showed that these IgA were able to inhibit NK cell-mediated ADCC by competition with IgG²².

Our study demonstrates that the change in formulation of vaccine antigens confers stronger protective immunity. The heterologous combination could be therefore particularly suitable for immunocompromised patients. Moreover, the confirmation that heterologous vaccination works effectively opens up new perspectives for applying vaccination to a larger number of people worldwide, thanks to the greater flexibility it allows. Finally, it would be interesting to test whether the use of a Chad boost as a third dose in at-risk patients having received a homologous BNT vaccination schedule (which represents the majority of individuals in Europe) would improve immunity in comparison to a third dose of BNT.

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Table I: Infection rate in vaccinated health care workers at the Lyon university hospital

Naive healthcare workers		BNT/BNT	Chad/BNT
Vaccinated subjects	Numbers of subjects	10216	2612
	Age- median [min-max]	43 [19-78]	31 [19-64]
SARS-CoV-2 qRT-PCR positive at least 14 days after second vaccine dose	Numbers of subjects (%)	33 (0.32)*	1 (0.04)*
	Age- median [min-max]	46 [22-61]	28

Table 2: clinical characteristics of patients in the Covid-ser-vac study

	Heterologous vaccine (n=29)	Homologous vaccine (n=31)	p-value	Adjusted p- value
Male sex. n (%)	9 (31.03)	8 (25.80)	0.7742	0.7742
Age. 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 consumption*. Weekly. n(%)	7 (24.13)	5 (16.12)	0.5269	0.6251
Delay between two vaccine doses median [IQR]	85 [84-85]	29 [26-31]	<0,0001	0.0007
Delay between last blood sampling and end of vaccine schedule median [IQR]	30 [28;34]	28 [27;31]	0,0245	0.057
Presence of Comorbidity*. n (%)	0	0	N/A	N/A

Comorbidities includes : Neurological disorders, ,cardiovascular disorders, hypertension, heart failure, diabetes, immune deficiency, liver disease, kidney disease, cancer, hypothyroidy, rheumatic disease

314

315 **Figure legends**

316

317 **Figure 1: Incidence of SARS-CoV-2 infection after different vaccination**
318 **regimens.** Graph data show the cumulative incidence of infection (as documented by
319 positive SARS-CoV-2 RT-PCR) among groups of health care workers HCWs that
320 were vaccinated with the homologous BNT/BNT combination within the
321 recommended 4-week timeframe between the two doses, or with the BNT boost after
322 receiving the first Chad dose approximately 12 weeks before, as recorded by the
323 service of occupational medicine, Hospices Civils de Lyon. Data show the infection
324 that occurred only after a 14-days period post boost, until the end of the recording
325 period. Clinical data and statistics are available in Table 1.

326

327 **Figure 2: Heterologous vaccination induces higher neutralizing antibody titers.**
328 Sera from Chad/BNT or BNT/BNT individuals were assayed for S1-specific IgG (A).
329 RBD-specific IgGs (B) or S1-specific IgA (C) using commercial Elisa kits, at different
330 times during the vaccination process (A-B) as indicated, or 4 weeks after full
331 vaccination (C). Data show the concentration (ng/ml) of immunoglobulins, and are
332 expressed as dot plots, one dot corresponding to one patient (n=30 in each group).
333 Statistical significance is shown (Mann-Whitney test, ***p < 0.01, **p < 0.01, *p <
334 0.05). (D-E) Sera from Chad/BNT or BNT/BNT individuals were assayed for their
335 capacity to neutralize the entry of virus-like particles pseudotyped with Wuhan-strain
336 SARS-CoV-2 envelope (D) or for their capacity to neutralize the infection of Vero E6
337 cells by different SARS-CoV-2 strains (E), as indicated. Data show the % of
338 neutralization, relative to a positive control (D) or the PRNT₅₀ (E), and are expressed

as dot plots(D) or violin plots (E) showing the mean, one dot corresponding to one patient (N=30 in each group). Statistical significance is shown between both groups for each condition (* symbols) or within groups between conditions (# conditions) using the Mann-Whitney test (***p < 0.01, **p < 0.01, *p < 0.05).

Figure 3. Analysis of RBD-specific memory B cells and Spike-specific memory T cells in vaccinated individuals.

(A-E) Flow cytometry analysis of Spike specific memory B cells. (A) Frequencies of SARS CoV 2 RBD-binding mBCs among the pool of polyclonal mBCs. (B and C) Proportions of unswitched (B) and switched (C) RBD-binding mBCs in % of RBD-binding mBCs. (D) Pattern of Ig isotypes expressed by switched mBCs (E) Proportions of resting (CD21⁺CD11c⁻) and activated (CD21⁻CD11c⁺/ CD21⁻CD11c⁻/ CD21⁺CD11c⁺) mBCs among the entire pool of RBD-binding mBCs. (F-H) T cell response. (F) IFN γ release after whole blood stimulation with RBD peptides. IFN γ was measured by Elisa in the supernatant. (G-H) Flow cytometry analysis of the percentage of cells positive for intracellular IFN γ expression among CD4 (G) or CD8 (H) T cells after PBMC stimulation with commercial Spike peptides. Data are expressed as dot plots of n = 30 patients and show means values. Statistical significance is shown using the Mann-Whitney test (***p < 0.01, **p < 0.01, *p < 0.05).

Methods

Cohort description

Sixty naïve HCWs for COVID-19 vaccinated with Pfizer BNT162b2 and/or Chad vaccines were included in a prospective longitudinal cohort study conducted in the Hospices Civils de Lyon (Lyon, France). Blood sampling was performed before

vaccination, before the second dose of vaccine and 4 weeks after the end of vaccination schedule. Absence of previous infection of SARS-CoV-2 was confirmed with Wantai Ab total assay in the pre-vaccinal sample. Demographic characteristics and delays between doses were depicted in Table 1 and Supplementary Figure 1. Written informed consent was obtained from all participants; 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), and the study was registered on ClinicalTrials.gov (NCT04341142).

Measurement of IgG titers

Sera were immediately stored at -80°C after blood sampling. RBD-specific IgG, S1-specific IgG were measured using the Siemens Atellica IM SARS-CoV-2 IgG (sCOVG), bioMerieux Vidas SARS-CoV-2 IgG diagnosis kits respectively, according to manufacturer's recommendations. For standardization of these assays to the first WHO international standard, the concentrations were transformed in BAU/mL using the conversion factors provided by the manufacturers.

Measurement of spike-specific IgA

ELISAs to evaluate the IgA binding to SARS-CoV-2 spike were performed as previously described²³. High binding 96-half-well plates (#2310M; NUNC) were coated with 100 µl per well of a spike protein solution (1 µg/ml; #40591-V08H Spike S1-RBD Sino Biologicals) in PBS overnight at 4°C. Plates were washed six times with washing buffer containing 1× PBS with 0.05% Tween 20 (Sigma-Aldrich) and incubated with 170 µl of blocking buffer per well containing 1× PBS with 3% milk and

0.05% Tween 20 (Sigma-Aldrich) for 1 hour at room temperature. Immediately after blocking, recombinant anti-RBD IgA (B Cell Design #IB3C4 PV) or serum samples were added in PBS and incubated for 1 hour at 37°C. Plasma samples were assayed at a 1:100 starting dilution and seven additional threefold serial dilutions. Recombinant anti-RBD IgA was used to perform a calibration curve starting at 1.5 µg/ml. Plates were washed six times with washing buffer and then incubated with anti-human IgA (A0295; Sigma-Aldrich) secondary antibody conjugated to horseradish peroxidase (HRP) in blocking buffer at 1:10000. Plates were developed by addition of the HRP substrate, 3,3',5,5"-tetramethylbenzidine (TMB; 34021; Thermo Fisher Scientific), for 10 min, and then 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 ICU COVID patients, diluted 200-fold in PBS) and negative control historical serum samples were added in duplicate to every assay plate for validation. The average of its signal was used for normalization of all the other values on the same plate with Excel software.

Live virus neutralization experiments

A plaque reduction neutralization test (PRNT) was used for the detection and titration of neutralizing antibodies. A 10-fold dilution of each serum specimen in culture medium was first heated for 30 min at 56 °C to avoid complement-linked reduction of the viral activity. Serial 2-fold dilutions (tested in duplicate) of the serum specimens in culture medium were mixed at equal volume with the live SARS-CoV2 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.

The plates were incubated at 37 °C in a 5% CO₂ atmosphere. The reading was evaluated microscopically 5 to 6 days later when the cytopathic effect of the virus control reached 100 to 500 TCID₅₀/150 µL. Neutralization was recorded if more than 50% of the cells present in the well were preserved. The neutralizing titer was expressed as the inverse of the higher serum dilution that exhibited neutralizing activity; a threshold of 20 was used (PRNT₅₀ titer ≥ 20). All experiments were performed in a biosafety level 3 laboratory. The different viral strains that were used were sequenced, and deposited 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)).

Monitoring of T cell response using whole blood IFN γ release assay

Fresh blood collected in heparinized tubes was stimulated for 22 hours at 37°C under 5% of CO₂ with SARS-CoV-2 peptide pools (derived from prototype Wuhan strain, NC_045512.2) targeting RBD (46 peptides, [C]= 8 µg/mL), (bioMérieux, France) diluted in IGRA solution (bioMérieux, France). The peptides (15-mer) encompassed the whole protein sequence and overlapped by 5-residues. The IGRA solution was used as a negative control and a mitogen as a positive control. The concentration of IFN γ in the supernatant was measured using the VIDAS automated platform (VIDAS® IFN γ RUO, bioMérieux). The measuring range was 0.08 to 8 IU/mL and WB IGRA positivity thresholds were defined at 0.08 IU/mL. The IFN γ response was defined as detectable when the IFN γ concentration of the test was above threshold and the negative control was below threshold or when the IFN γ concentration of the test minus IFN γ concentration of the negative control was above threshold.

440

441 **Monitoring of T cell responses by flow cytometry**

442 Overnight-rested PBMCs were stimulated with SARS-CoV-2 overlapping peptides
443 pools against SARS-CoV-2 Spike protein (PepTivator, Miltenyi Biotec) at a final
444 concentration of 1 µg/ml for 1 h in the presence of 1 µg/ml monoclonal antibodies
445 CD28 and CD49d, and then for an additional 5 h with GolgiPlug and GolgiStop (BD
446 Biosciences). Dead cells were labelled using Fixable Viability eFluor 780 dye
447 (ThermoFischer Scientific). Surface markers, including BV786-conjugated anti-CD3
448 (BD Biosciences), BUV486- conjugated anti-CD4 (BD Biosciences), PE-Cy7-
449 conjugated anti-CD8, APC-Cy7-conjugated anti-CD14, APC-Cy7-conjugated anti-
450 CD16 and APC-Cy7- conjugated anti-CD19 (all BioLegend) were stained. Cells were
451 then washed, fixed with Cytofix/Cytoperm (BD Biosciences) and stained with PE-
452 conjugated anti-IFN γ (BioLegend). Negative controls without peptide stimulation were
453 run for each sample. All samples were acquired on a BD LSRFortessa (BD
454 Biosciences) flow cytometer and analyzed using FlowJo version 10 software.

455

456 **SARS-CoV-2 pseudoparticle preparation and neutralization**

457 SARS-CoV-2 spike-pseudotyped murine leukemia virus (MLV) retrovirus particles
458 were produced as we described for SARS-CoV ²⁴. Briefly, HEK293T cells (ATCC
459 CRL-1573) were transfected with constructs expressing MLV Gag-Pol, the green
460 fluorescent protein (GFP) reporter, and the SARS-CoV-2 spike (a kind gift from D.
461 Lavillette). A plasmid encoding the spike protein harboring the D614G mutation was
462 generated by PCR mutagenesis. Control pseudoparticles pseudotyped with the
463 unrelated RD114 virus surface glycoprotein (from a cat endogenous virus) was
464 generated as previously described ²⁵. For neutralization assays, a sample of $\sim 1 \times 10^3$

pseudoparticles was incubated with a 100-fold dilution of sera or control antibodies for 1h at 37 °C, spinoculated for 2h at 2.500g before infection of Vero-E6R cells. At 72 h post infection, the percentage of GFP-positive cells was determined by flow cytometry. As a control, the same procedure was performed using RD114 pseudoparticles. Anti-spike SARS-CoV-2 RBD (Sino Biological) and anti-gp70 RD114 (ViroMed Biosafety Labs) antibodies were used as positive and negative control nAbs, respectively.

Generation of fluorescent SARS-CoV-2 RBD tetramers.

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

Flow cytometric analysis of SARS-CoV-2 RBD-specific B cells.

Cryopreserved PBMCs were centrifuged and resuspended in PEB Buffer (PBS + 0.5% BSA and 2 mM EDTA) and incubated with Fc receptor block (Miltenyi 130-059-901) 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 the following antibodies : anti-CD3-APC Fire 810 (Biolegend 344858), anti-CD11c-BV785 (Biolegend 301644), anti-CD19-PE Vio770 (Miltenyi 130-113-170), anti-CD20-BV421 (BD 562873), anti-CD21-BUV496 (BD 750614), anti-CD27-PercP-Vio700 (Miltenyi 130-113-632), anti-CD38-Viobright FITC (Miltenyi 130-113-433), anti-IgM-PE-CF594 (BD 562539), anti-IgD-BV605 (Biolegend 348232) and anti-IgG-BV480 (BD 746341) together with both the PE- and APC-conjugated recombinant RBD tetramers. 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 at 4% paraformaldehyde (PFA) for 20 min at 4°C in the dark before a final wash and resuspension for analysis. Cells were then acquired on a Cytex Aurora spectral flow cytometer equipped with five lasers operating on 355nm, 405nm, 488nm, 561nm and 640nm. Data were analyzed using FlowJo10 software (Becton Dickinson).

Statistical analysis

GraphPad Prism version 8 was used for statistical analysis, curve fitting and linear regression. A two-tailed Mann-Whitney U test was used to calculate p values for continuous, non-parametric variables. For comparing more than one population, Kruskal-Wallis testing was used with Dunn's multiple comparison testing.

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516

517 **Author contributions**

518 B Pozzetto, V Legros, S Djebali, V Barateau, M Villard, L Peyrot, M Yaugel-Novoa, S
519 Denolly, B Boson, T Bourlet, M Valette performed experiments and analyzed the data.
520 T Andrieu helped with the analysis of flow cytometry data. , O Allatif performed
521 statistical analyses. N Guibert, JB Fassier, A Massardier-Pilonchéry analyzed the
522 clinical infection data. B Lina and FL Cosset supervised some of the experimental
523 tasks. S Paul, T Defrance, J Marvel, T Walzer, Sophie Trouillet-Assant designed the
524 study, analyzed the data, and wrote the manuscript.

525

526 **Competing interest declaration**

527 Karen Brengel-Pesce is a Biomérieux employee

528

529

530 **Additional information**

531 Supplementary Information is available for this paper. Figure S1 shows an outline of
532 the immunological study. Figure S2 shows the B cell gating strategy as well as flow
533 cytometry plots of data concatenated from all patients. Figure S3 shows a correlation
534 matrix of all immune parameters.

535

536 **Extended data figure legends**

537

538 **Figure S1: study design**

539

540 **Figure S2. Analysis of RBD-specific memory B cells.** (A) Gating strategy. From
541 left to right, the first three pseudo-colour plots show the successive gateings applied
542 for single cells and viable cells. B cells were gated as CD19⁺/CD3⁻ cells (4th plot).
543 Within the B cell gate, memory B cells (mBCs) were defined as non-naïve/non-
544 transitional B cells on the same biparameter plot (6th plot). Second line: examples of
545 the staining patterns of the PE- (RBD#1) and APC- conjugated (RBD #2) SARS-
546 CoV2 RBD probes in B cell gate (left) and memory B cell gate (right). Third line:
547 gating strategy to define unswitched (IgD⁺CD27⁺), and switched (csM, IgD⁻CD27⁺)
548 RBD-specific memory B cells. Middle: gating strategy to define IgG and IgG⁻/IgM⁻
549 switched RBD-binding memory B cells based on the IgM/IgG stainings.

550 (B-E) Concatenated phenotypic profiles of RBD-specific memory B cells for all
551 individuals in the ChAd/BNT and BNT/BNT groups. For each group, the phenotypic
552 profiles correspond to the concatenation of the FACS analysis data collected from 30
553 individual vaccinees. (B) Visualization of RBD-binding B cells in the memory B cell

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 switched 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.

Figure S3: analysis of inter-relationships between immune parameters.

A matrix file with all immunological measurements for all vaccinated individuals was created and Spearman correlations were calculated. The size and the color of the circles correspond to the correlation, as indicated, and the stars indicate the significance of the correlation.

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Abbreviations: Chad: AstraZeneca ChadOx1 nCoV-19 vaccine; BNT: Pfizer BNT162b2 vaccine; VOC: variant of concern; VNT: virus neutralization test; PRNT: plaque reduction neutralization test; RBD: receptor binding domain;

Figure 1

Figure 2

Figure 3

Figure S1

Figure S2

A

B

C

D

E

Chad/BNT

Figure S3