Supplementary appendix

Methods

Patients

The prospective COVADIS study (NCT04870411) was conducted in patients with systemic inflammatory diseases managed in the Internal Medicine department from Cochin Hospital, University of Paris (Paris, France). Healthcare immunocompetent workers from the same hospital were included as controls. Patients with a positive Covid-19 serology at baseline (day 0) were excluded from the main analysis. Cases and controls received the BNT162b2 mRNA vaccine from BioNTech/Pfizer according to the recommendations of the French National Authority for Health, considering either their immunocompromised status or their occupational exposure risk. Ethics approval was obtained by Comité de Protection des Personnes Nord-Ouest II. Cases and controls provided written informed consent.

Clinical and laboratory data

Clinical data were collected at baseline and during follow-up until month 3, and included demographics, underlying disease, disease activity, renal involvement, current and previous (within the 12 months before the first dose of vaccine) therapies. Total lymphocytes count and immunoglobulins dosage in peripheral blood were also collected at baseline. To evaluate vaccine immunogenicity, blood samples were collected before the first dose of vaccine (M0), before the second dose (M1), 3 and 6 months after the first dose (M3-M6).

T and B cell immunophenotyping

Briefly, after 2 washes with PBS 1X, FcR Blocking Reagent (Miltenyi) was used according to manufacturer instructions. Cellular stainings were performed at 4°C for 30 min protected from light. Antibodies used are summarized in the **Supplementary Table 2**. Samples were acquired using the BD LSRFortessa™ X-20 Cell Analyzer. Data were analyzed using FlowJo software

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v10.6. Gating strategies for immunoprofiling of B cell and T cell subpopulation compositions are shown in **Supplementary Figures 1-2**.

S-Flow assay

The S-Flow assay was adapted using 293T cells stably expressing the S protein (293T Spike cells) and 293T empty cells as control[1]. Cells were incubated at 4°C for 30 min with sera (1:300 dilution) in PBS containing 0.5% BSA and 2 mM EDTA. Cells were then washed with PBS, and stained using an anti-human IgG Fc Alexafluor 647 antibody (109-605-170, Jackson ImmunoResearch) and an anti-human IgA Alpha chain Alexafluor 488 antibody (109-545-011, Jackson ImmunoResearch). After 30min at 4°C, cells were washed with PBS and fixed for 10 min using 4% paraformaldehyde (PFA). Binding units (BU) were calculated to standardize the results. A standard curve with serial dilutions of a human anti-S monoclonal antibody (mAb48) was acquired in each assay. The logarithm of the median of fluorescence of each sample was reported on the curve to obtain an equivalent value (in ng/mL) of mAb48 concentration in logarithm [1,2]. Data were acquired on an Attune NxT instrument (Life Technologies). Flow cytometry data were analyzed with FlowJo v.10 software (TriStar). Calculations were performed using Excel 365 (Microsoft).

The specificity and sensitivity of the S-Flow serological assay were originally assessed with the Wuhan S protein using 253 pre-pandemic samples and 377 RT–qPCR-confirmed SARS-CoV-2 samples. The sensitivity is 99.2% with a 95% confidence interval of 97.69–99.78% and the specificity is 100% (98.5–100%)[2,3].

To better characterize the S-Flow assay, we run a series of vaccinated (n=144) and convalescent (n=59) individuals that we characterized in previous studies[4,5] using two commercially available ELISA (Abbott 147 and Beckmann 56, see details in **Supplementary Table 3**). We also included the two WHO international reference sera, (20/136) arbitrary assigned at 1000 BAU/ml and (20/130) calculated to be 502 BAU/ml[6]. We observed a strong correlation

(R=0.73; p<0.0001) between the BU (Binding Units determined by S-Flow, corresponding to anti-spike IgG) and the BAU/mL (Binding Antibody Units) obtained with the commercial ELISAs (**Supplementary Figure S3A)**. We then performed a Passing-Pablok regression on all samples, which shows that the relationship between BU and BAU/mL is linear (BAU/mL = 0.35 [0.126-0.534] + 0.892 [0.791-1,001] x BU) (**Supplementary Figure S3B**). We used this formula to calculate equivalent BAU/mL (eqBAU/mL) from our BU data. Finally, we performed serial two-fold dilution of the WHO reference sera 20/136, to show that our S-Flow assay is linear in between 1000 and 15 BAU/mL (**Supplementary Figure S3C**).

After testing 253 pre-pandemic samples, the positivity of a sample was defined as a specific binding above 40%. The specific binding was calculated as follows: 100 × (percentage binding 293T S protein − percentage binding 293T-empty)/(100 − percentage binding 293T-empty). In this cohort, uninfected and pre-vaccinated samples were below 40% with BU equivalent to maximum 1.1.

Virus strains

The B.1.1.7 (Alpha) variant originated from an individual in Tours (France) returning from the United Kingdom. The B.1.617.2 (Delta) variant originated from a hospitalized patient in Paris returning from India[4]. Both patients provided informed consent for the use of the biological materials. The variant strains were isolated from nasal swabs using Vero E6 cells and amplified by two passages. Titration of viral stocks was performed on Vero E6 cells, with a limiting dilution technique allowing a calculation of the 50% tissue culture infectious dose, or on S-Fuse cells. Viruses were sequenced directly on nasal swabs and after two passages on Vero cells.

S-Fuse neutralization assay

U2OS-ACE2 GFP1–10 or GFP 11 cells, also termed S-Fuse cells, were used, these cells becoming GFP+ cells when productively infected with SARS-CoV-2[4]. Cells were mixed (at a

1:1 ratio) and plated at 8.10^3 cells per well in µClear 96-well plates (Greiner Bio-One). SARS-CoV-2 strains were incubated with sera at the indicated concentrations for 15 min at room temperature and added to S-Fuse cells. 18 h later, cells were fixed with 2% paraformaldehyde, washed and stained with Hoechst (1:1,000 dilution; Invitrogen). Serial dilutions of the sera were performed to be tested starting at a first dilution of 1:30. Sera with no detectable neutralizing activity at this first dilution were thus defined as "neutralizers". Images were acquired with an Opera Phenix high-content confocal microscope (PerkinElmer). The number of syncytia and nuclei were quantified using the Harmony software (PerkinElmer). The percentage of neutralization was calculated using the number of syncytia and the following formula: 100 x (1 − (value with serum – value in 'noninfected')/(value in 'no serum' − value in 'noninfected')). Neutralizing activity of each serum was expressed as the half maximal effective dilution (ED50), calculated using a reconstructed curve of neutralization at each concentration. Sera were heat-inactivated for 30 min at 56°C before use.

T-cell response using enzyme-linked immunoSpot (EliSpot)

Peripheral blood mononuclear cells (PBMCs) were isolated from fresh blood. After density gradient separation, lymphocytes were enumerated by flow cytometry using the BD Tritest™ CD3FITC/ CD8PE/ CD45PerCP and BD Trucount™ Tubes (BD Biosciences, Le Pont de Claix, France). The EliSpot assay was conducted as previously described . Briefly, day 0, sterile PVDF strips (Millipore, Saint-Quentin-en-Yvelines, France) were coated overnight at 4°C with an IFN-γ antibody (U-CyTech, Utrecht, Netherlands). Day 1, strips were blocked with culture medium 1 hour and then, PBMCs were seeded at 200 000 CD3+T cell/well in duplicates and stimulated for 18-20hrs with individual pool of 15-mer peptides with 11 amino acids overlap at a final concentration of 10 µmol/L. Day 2, PBMCs were removed and IFN-γ secretion was revealed using a biotin-conjugated IFN-γ antibody (U-CyTech), streptavidin-horseradish peroxidase (UCyTech) and 3-amino-9-ethylcarbazole (AEC) (U-CyTech). Spots were

enumerated using an automated EliSpot reader (Autoimmune Diagnostika (AID) reader, Strassberg, Germany). To identify SARS-CoV-2-spike-specific T cells, we used a commercially available pool derived from a peptide scan through SARS-CoV-2 (JPT Peptide Technologies GmbH, BioNTech AG, Berlin, Germany). The S1 domain is specific to SARS-CoV-2 and also includes RBD region whereas the S2 region is the most conserved region between various coronaviruses including common cold coronavirus. Results were expressed as Spot Forming Unit (SFU)/106 CD3+ T lymphocytes after subtraction of background values from wells with non-stimulated cells. The EliSpot was technically validated when the mean number of spots in unstimulated wells was under than or equal to 10. The detection threshold was set at 3SD above the average basal reactivity (mean spot number in RPMI wells), fixed at a minimum of 11 SFU/10^6 CD3+ to rule out false positives where the background was very low.

Statistical analysis

No statistical methods were used to predetermine sample size. The experiments were performed in blind regarding to the allocation groups. Flow cytometry data were analyzed with FlowJo v.10 software (TriStar). Calculations were performed using Excel 365 (Microsoft). Figures were drawn using GraphPad Prism 9. Statistical analyses were conducted using GraphPad Prism 9. Statistical significance between different groups was calculated using the tests indicated in each figure legend. To assess clinical data and treatment associated with cross-neutralization of Alpha and Delta variants, and T-cell response at 3 months, patients were compared using *t*-tests for quantitative variables and analyses of variance for qualitative variables. Associations between both quantitative humoral and T-cell response (defined by neutralization titers for both viruses, and the number of circulating S1 and S2 peptide pool SARS-CoV-2 specific IFNγ-producing T cells) and clinical data were assessed by multivariate

linear regression models. Those analyses were performed using R version 3.6.1. (R Foundation for Statistical Computing, Vienna, Austria).

Tables

Table S1. Immunological features at baseline

Median (IQR)	Control n= 17	Rituximab n=22	Methotrexate n=16	Immunosuppressive drugs n=19	Others n=7
Lymphocytes (/mm^3)	2400 (2125-2575)	1100 (900-1780)	1200 (110-2200)	1570 (1100-1930	1600 (848-2125)
CD19+ B cells (/mm3)	166 (105-210)	0.1 (0-0.3)	63 (49-203)	180 (68-355)	88 (29-141)
Naive B cells (%)	67 (40-78)	-	47 (18-70)	73 (49-91)	52 (51-74)
Switch memory B cells (%)	14 (8-25)	-	23 (11-33)	7 (2-27)	18 (15-31)
CD4+ T cells (/mm3)	61 (56-65)	51 (42-72)	54 (47-67)	58 (50-74)	55 (41-69)
Naïve CD4+ T cells (%)	41 (31-55)	38.5 (19-53)	31 (13-61)	46 (35-56)	24 (17-46)
Memory C4+ T cells (%)	52 (39-61)	55 (37-74)	65 (33-82)	48 (39-58)	71 (50-80)
Follicular helper CD4+ T cells (%)	8 (6-10)	7 (5-9)	7 (5-8)	8 (6-13)	9 (6-12)
CD8+ T cells (%)	33 (28-36)	37 (23-46)	39 (29-41)	31 (23-41)	34 (25-46)
Immunoglobulins, g/L					
IgG	11.1 (9.9-12.4)	7 (6-8.6)	10.4 (7.9-13.3)	12.4 (8.6-18)	11.6 (10.2-12.9)
IgA	2.2 (1.5-2.6)	1.4 (0.9-2)	1.9 (1.5-2.8)	1.7 (1.4-2.5)	2.1 (1.8-3)
IgM	0.9 (0.7-1.3)	0.4 (0.2-0.8)	0.9 (0.7-1.3)	1 (0.5-1.3)	0.8 (0.6-2.5)
IgG1	5.2 (4.2-6.2)	3.8 (2.8-4.4)	5.9 (3.6-7.3)	5.4 (4.2-10.5)	5.3 (4.6-6.1)
IgG2	3.5 (2.4-4.7)	1.6 (1.3-2.1)	2.4 (1.6-3.4)	3.4 (2.2-4.2)	3.4 (1.8-3.9)
IgG3	0.5 (0.3-0.6)	0.3 (0.2-0.5)	0.5 (0.3-0.7)	0.8 (0.4-1.3)	0.6 (0.3-0.7)
IgG4	0.55 (0.3-0.7)	0.2 (0.1-0.4)	0.15 (0.03-0.3)	0.3 (0.1-0.4)	0.3 (0.1-0.6)

Table S2. Antibodies used for T and B cell immunophenotyping.

	Marker	Fluorochrome	Clone	Supplier	Cat. number
Panel B	CD19	APC	SJ25C1	Biolegend	363006
	IgD	PE	I-A62	BD Pharmigen	555779
	CD21	FITC	BL13	Beckman Coulter	IMO473U
	CD27	BV510	O323	Sony	2114180
	CD24	BV421	ML5	BD Horizon	562789
	CD38	PE-Cy7	HB-7	Biolegend	356608
Panel T	CD3	PerCP-Cy5.5	OKT3	Biolegend	317336
	CD4	BV510	OKT4	Biolegend	317444
	CD8	APC	HiT8a	Biolegend	300912
	CD45RA	AF488	Hi100	Biolegend	304114
	CD38	PE-Cy7	HB-7	Biolegend	356608
	CCR6	BV650	G034E3	Biolegend	353426
	CXCR5	BV711	J252D4	Sony	2384665
	PD-1	PE	EH12.2H7	Biolegend	329906
	CXCR3	PE-Dazzle	G025H7	Biolegend	353736
	ICOS	AF700	C398.4A	Sony	2167640

Table S3. Characteristics of commercial ELISA

	Bekcman Coulter	**Abbott**
Exact name	Access SARS-CoV-2 IgG (1st IS)	SARS-CoV-2 IgG II Quant
Spike antigen used	Paramagnetic particles coated with recombinant SARS-CoV-2 protein specific for the receptor binding domain (RBD) of the S1 protein.	Paramagnetic particles coated with recombinant SARS-CoV-2 protein specific for the receptor binding domain (RBD) of the S1 protein.
Type	Quantitative	Quantitative
Measurement range	8 BAU/mL to 36 000 BAU/mL	6.8 BAU/mL to 80 000 BAU/mL
LoQ, 8 BAU	8 BAU/mL	6.8 AU/mL
Cut-offs for positivity	30 BAU/mL	50 AU/mL
Units, conversion to BAU	UI/mL x1 = BAU/mL	0.142 x AU/mL x1 = BAU/mL
Instrument used to measure	UniCel DxI Beckman Coulter	Architect I System

Table S4. Characteristics of patients according to the neutralization of alpha and delta variants and to the T-cell response (n=82).

	Neutralization of alpha variant			Neutralization of delta variant			T-cell response		
	No	Yes	*P*	No	Yes	*P*	No	Yes	*P*
N	30	52		39	43				
Age, years	55.47 (16.64)	50.13 (15.42)	0.147	2.56 (17.26)	51.65 (14.94)	0.798	48.05 (17.90)	53.96 (15.31)	0.169
Male	9 (30.0)	14 (26.9)	0.965	23 (28.0)	11 (28.2)	1.000	5 (26.3)	15 (27.3)	1.000
Treatment group			<0.001			<0.001			0.036
Controls	0 (0.0)	21 (40.4)		0 (0.0)	21 (48.8)		1 (5.3)	18 (32.7)	
Immunosuppressants	9 (30.0)	10 (19.2)		11 (28.2)	8 (18.6)		6 (31.6)	11 (20.0)	
Methotrexate	2 (6.7)	13 (25.0)		7 (17.0)	8 (18.6)		6 (31.6)	5 (9.1)	
Rituximab	19 (63.3)	1 (1.9)		20 (51.3)	0 (0.0)		5 (26.3)	15 (27.3)	
Other	0 (0.0)	7 (13.5)		1 (2.6)	6 (14.0)		1 (5.3)	6 (10.9)	
Group of disease			<0.001			<0.001			0.115
Controls	0 (0.0)	21 (40.4)		0 0.0)	21 (48.8)		1 (5.3)	18 (32.7)	
CTD	9 (30.0)	20 (38.5)		14 (35.0)	15 (34.9)		9 (47.4)	17 (30.9)	
Vasculitis	20 (66.7)	5 (9.6)		22 (56.4)	3 (7.0)		7 (36.8)	17 (30.9)	
Other	1 (3.3)	6 (115)		3 (7.7)	4 (9.3)		2 (10.5)	3 (5.5)	
Active disease (N=61)	9 (30.0)	7 (22.6)	0.713	12 (30.8)	4 (9.3)	<0.001	7 (38.9)	6 (16.2)	0.129
Glucocorticoids (%)	21 (70.0)	22 (42.3)	0.029	27 (69.2)	16 (37.2)	0.007	15 (78.9)	23 (41.8)	0.012
Lymphocytes, *per mm3*	1340.36 (725.59)	1618.36 (690.38)	0.126	1434.59 (745.91)	1580.48 (671.36)	0.429	1418.89 (731.18)	1544.68 (714.65)	0.542
IgG, g/L	9.34 (5.70)	12.41 (5.81)	0.025	10.74 (7.47)	11.72 (3.85)	0.470	13.18 (10.41)	10.45 (3.22)	0.094
IgA, g/L	1.46 (0.67)	2.38 (1.50)	0.002	1.82 (1.60)	2.23 (0.93)	0.175	2.02 (1.25)	2.10 (1.42)	0.830
IgM, g/L	0.69 (0.61)	1.06 (0.61)	0.012	0.78 (0.64)	1.06 (0.60)	0.047	0.89 (0.61)	0.90 (0.66)	0.950
IgG1, g/L	5.11 (3.64)	6.12 (3.21)	0.204	5.74 (4.13)	5.73 (2.50)	0.990	7.05 (5.75)	5.15 (1.82)	0.037
IgG2, g/L	2.05 (1.13)	3.53 (1.78)	<0.001	2.58 (1.98)	3.34 (1.31)	0.051	2.95 (2.53)	3.00 (1.36)	0.915
IgG3, g/L	0.56 (0.59)	0.58 (0.28)	0.814	0.63 (0.56)	0.52 (0.21)	0.263	0.70 (0.65)	0.50 (0.23)	0.065
IgG4, g/L	0.21 (0.18)	0.36 (0.30)	0.019	0.21 (0.18)	0.39 (0.32)	0.003	0.20 (0.21)	0.33 (0.30)	0.098

Data are presented as mean (SD) for continuous variables and as count (percent) for qualitative variables. Comparisons between patients with humoral or cellular response were compared using t-tests for continuous variables and analyses of variance for qualitative variables.

Figure legends

Figure S1. Gating Strategy for Immunoprofiling of B cell Subpopulation Compositions.
Lymphocytes were first gated based on their FSC-A versus SSC-A. CD19+ B cells were separated between naive (CD27-) and memory (CD27+) subsets then IgD marker helped defined switched memory (CD27+IgD-), marginal zone (CD27+IgD+) and naive (CD27-IgD+) CD19+ B cells. Plasmablasts were defined as CD24-CD38high among memory CD19+ B cells.

Figure S2. Gating Strategy for Immunoprofiling of T cell Subpopulation Compositions.
Lymphocytes were first gated based on their FSC-A versus SSC-A. Within the CD3+ T cell compartment, CD4+ T cells were further subdivided into naïve (CD45RA+) and memory (CD45RA-). Among memory CD4+ T cells, PD-1 and CXCR5 surface markers were used to define follicular helper T cells (TFH, CXCR5+PD-1+CD45RA-CD4+). Identification of Th1 (CXCR3+CXCR6-), Th2 (CXCR3-CXCR6-), Th17 (CXCR3-CXCR6+) and Th1+Th17+ double positive (CXCR3+CXCR6+) populations were identified among CXCR5-CD45RA-CD4+ subset.

Figure S3. Comparison between the S-Flow assay and two commercially available ELISA.
A. Correlation between the S-Flow assay and two commercially available ELISA (Abbott and Beckmann) using sera from vaccinated (left panel; n=144) and convalescent (right panel; n=59) individuals that we characterized in previous studies. We observed a correlation (R=0.73; $p<0.0001$) between the BU (Binding Units determined by S-Flow, anti-spike IgG) and the BAU (Binding Antibody Units obtained with the commercial ELISAs and measuring the anti-RBD IgG).
B. Passing-Bablok linear regression between the BU obtained with the S-Flow assay and BAU/mL obtained with commercial assays. Sera from vaccinated (n=144) and convalescent

individuals (n=59) were pooled for the analysis. The red line indicates the linear regression. Dotted lines show the 95% confidence interval. Colored squares depicted values obtained with WHO/reference sera 20/136 (1000BAU/mL) and 20/130 (502 BAU/mL)

C. Linearity of S-Flow. S-Flow BU values of two-fold serial dilutions of the WHO refence serum 20/136 (1000/BAU/mL). The red line indicates the result of an ordinary linear regression.

Figure S4. Laboratory Findings in Patients Included in the Study.

A. Lymphocyte counts, absolute number of CD3+ T cells, CD19+ B cells and proportions (frequencies) of CD19+ B cells, CD8+ T cells and CD4+ T cells among lymphocytes in peripheral blood according to the treatments received.

B. Concentration of immunoglobulin G, immunoglobulin A, immunoglobulin M proteins measured in serum. Data indicate median. Each dot represents a single patient. Two-sided Kruskal-Wallis test with Dunn's test for multiple comparisons between group of treatment was performed with median reported; *P < 0.05; **P < 0.01; ***P < 0.001.

Figure S5. Immunoprofiling of Peripheral Blood Mononuclear Cells at Baseline.

Proportions (frequencies) in peripheral blood of naive B cells and switched memory B cells among CD19+ B cells, naïve CD4+ T cells and memory CD4+ T cells among CD4+ T cells, follicular helper CD4+ T cells and TH1 CD4+ T cells among CD4+ memory CD4+ T cells and CD8+ T cells among lymphocytes according to the treatments received. Data indicate median. Each dot represents a single patient.

Figure S6. Kinetics of Anti-spike IgG after BNT162b2 Vaccine.

A. Levels of anti-S IgG antibodies (defined as binding units; BU) before the first dose of vaccine (M0), before the second dose (M1) and after full vaccination at 3 months (M3) according to the treatments received. The dotted line indicates threshold (BU=1.1 for IgG).

Figure S7. Kinetics of TH1 CD4+ T cells, Follicular Helper CD4+ T cells, Plasmablasts and Switched Memory B cells After BNT162b2 Vaccine.

Proportions (frequencies) in peripheral blood of switched memory B cells among CD19+ B cells and follicular helper CD4+ T cells among memory CD4+ T cells before the first dose (M0), before the second dose (M1) and after full vaccination at 3 months (M3) according to the treatments received.

Figure S8. Cross-Neutralization of the Alpha and Delta Variants and Correlation with specific Anti-Spike IgG Levels at 3 Months.

A-B. Correlation between Alpha and Delta neutralization titers (ED50) (left panel) or between IgG levels and Alpha neutralization titer (right panel). **(A)** control group and **(B)** patient groups. A Spearman correlation model was applied. p and r^2 values are indicated.

Figure S9. Humoral Immune Response to SARS-CoV-2 in Convalescent Vaccinated Individuals.

A. Level of anti-S IgG antibodies at M3 post-vaccination in the control group (black dots) and cases groups (color dots) with a positive (+) or negative (-) serology before vaccination. The dotted line indicates threshold (BU=1.1 for IgG).

B. Neutralizing titers of sera against Alpha (left panel) and Delta (right panel) variants are expressed as ED50 values. The lower limit of detection (ED50=30) and the upper line of quantification (ED50=30,000) are indicated by dotted lines.

Two-sided Kruskal-Wallis test with Dunn's test for multiple comparisons was performed. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Figure S10. Kinetics of specific T-cell response against the SARS-CoV-2 S2 peptide pool.

Kinetic of specific T-cells responses against the SARS-CoV-2 S2 peptide before the first dose of vaccine (M0), before the second dose (M1) and after full vaccination at 3 months (M3) according to the treatments received. Data indicate median. Each dot represents a single patient.

Figure S11. Relationship Between Humoral and Cellular Immune Responses Against SARS-CoV-2.

Each dot represents the mean of all patients in the indicated groups. Bars are the standard deviation. X axis is the neutralization titer (ED50) for Alpha (left panel) or the levels of IgG (right panel). Y axis is the levels of S1-specific T-cell responses measured by ELISPOT. Dotted lines indicate the limit of detection.

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