SARS-CoV-2 Omicron Spike recognition by plasma from individuals receiving BNT162b2 mRNA vaccination with a 16-week interval between doses

Graphical abstract
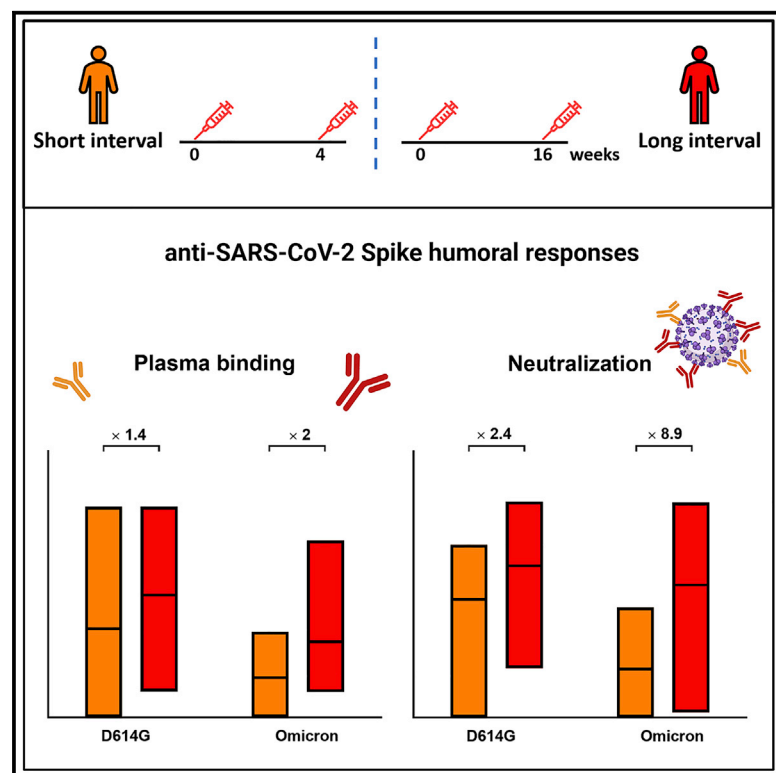
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In brief

Chatterjee et al. report that extending the interval between doses in naive individuals leads to better recognition and neutralization of the SARS-CoV-2 Omicron Spike than in those receiving a 4-week interval. Vaccinated convalescent individuals present higher responses than naive vaccinated individuals

Highlights

- A long interval between vaccine doses leads to good recognition of the Omicron Spike

- It also leads to detectable neutralization of the Omicron Spike

- These responses are stronger than in naive donors vaccinated with a short interval

- Vaccine-elicited responses in convalescent donors are superior than in naive donors

CellPress

Report

SARS-CoV-2 Omicron Spike recognition by plasma from individuals receiving BNT162b2 mRNA vaccination with a 16-week interval between doses

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SUMMARY

Continuous emergence of SARS-CoV-2 variants of concern (VOCs) is fueling the COVID-19 pandemic. Omicron (B.1.1.529) rapidly spread worldwide. The large number of mutations in its Spike raise concerns about a major antigenic drift that could significantly decrease vaccine efficacy and infection-induced immunity. A long interval between BNT162b2 mRNA doses elicits antibodies that efficiently recognize Spikes from different VOCs. Here, we evaluate the recognition of Omicron Spike by plasma from a cohort of SARS-CoV-2 naive and previously infected individuals who received their BNT162b2 mRNA vaccine 16 weeks apart. Omicron Spike is recognized less efficiently than D614G, Alpha, Beta, Gamma, and Delta Spikes. We compare with plasma activity from participants receiving a short (4 weeks) interval regimen. Plasma from individuals of the long-interval cohort recognize and neutralize better the Omicron Spike compared with those who received a short interval. Whether this difference confers any clinical benefit against Omicron remains unknown.

INTRODUCTION

SARS-CoV-2 variants are constantly evolving under immune selective pressure. Ongoing mutational events in the viral genome leads to the emergence of variants with unique properties, including increased transmission capabilities and resistance to antibodies elicited by both natural infection and vaccination. Based on transmission capabilities, virulence, and vaccine effectiveness, SARS-CoV-2 variants are classified as variants of concern (VOCs), variant of interest (VOIs), or variants under monitoring (VUMs) (WHO, 2021). In late 2020, the Alpha (B.1.1.7) variant emerged. The N501Y Spike mutation increased its affinity for the ACE2 receptor, leading to increased transmissibility (Davies et al., 2021; Prevost et al., 2021; Rambaut et al., 2020). The accumulation of E484K and K417N/T mutations along with N501Y in the receptor binding domain (RBD) led to the

emergence of Beta (B.1.351) and Gamma (P.1) lineages, which rapidly spread worldwide (Amanat et al., 2021; ECDC, 2021; Tang et al., 2021). In April 2021, the Delta (B.1.617.2) variant emerged and quickly spread to most countries (Allen et al., 2022; Planas et al., 2021b), but is rapidly being replaced by Omicron (B.1.1.529). The World Health Organization designated Omicron as a VOC on November 26, 2021(WHO, 2021). Omicron accumulated more than 30 mutations in its Spike, raising concerns about a major antigenic drift that could significantly decrease vaccine efficacy.

Here we evaluated the recognition of the Omicron Spike by plasma from a cohort of SARS-CoV-2 naive and previously infected individuals who received the two BNT162b2 mRNA vaccine doses 16 weeks apart. We compared these responses with those elicited in individuals receiving a short dose interval regimen (4 weeks). Plasma from vaccinated previously infected
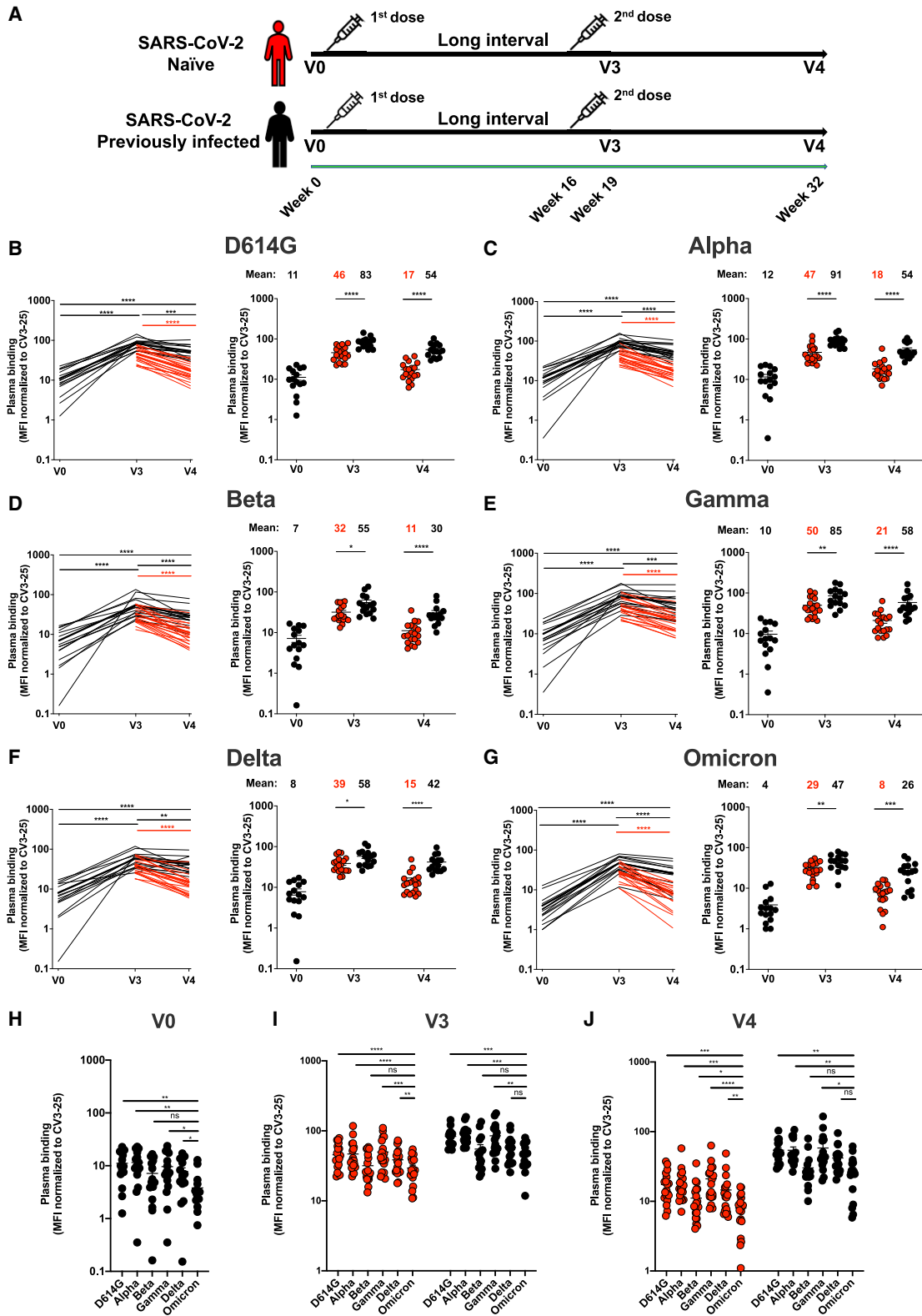
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individuals recognized more efficiently all tested Spikes (D614G, Alpha, Beta, Gamma, Delta, and Omicron) than those from naive vaccinated individuals. Omicron Spike was recognized less efficiently than D614G, Alpha, Beta, Gamma, and Delta Spikes. However, plasma from individuals receiving a long interval recognized and neutralized better the Omicron Spike compared with those who received a short interval.

RESULTS

Recognition of Spike variants by plasma from vaccinated individuals

The antigenic profile of D614G, Alpha, Beta, Gamma, Delta, and Omicron Spikes was assessed with plasma collected 3 weeks (V3) and 4 months (V4) after the second dose of the BNT162b2 mRNA vaccine administered with a 16-week interval between doses (Figure 1A) (Tauzin et al., 2022). Briefly, 293T cells were transfected with plasmids coding for full-length Spike variants. Two days post-transfection, cells were incubated with the indicated plasmas followed by flow cytometry analysis, as described (Anand et al., 2021; Beaudoin-Bussieres et al., 2020; Gasser et al., 2021; Prevost et al., 2020; Tauzin et al., 2021, 2022). Spike expression levels of VOCs were normalized to the signal obtained with the conformationally independent anti-S2 neutralizing CV3-25 antibody (Li et al., 2021; Prevost et al., 2021; Ullah et al., 2021) that efficiently recognized and neutralized all VOCs Spike, including Omicron (Figure S1). Using plasma from previously infected individuals or from naive double-vaccinated individuals, we observed a significant increase of recognition of all tested Spikes upon vaccination (Figures 1B–1G), in agreement with previous observations (Stamatatos et al., 2021; Tauzin et al., 2021, 2022). In all cases, the Omicron Spike was significantly less recognized than all other Spikes, with the exception of the Beta variant (Figures 1D, 1H–1J). At V3 and V4, levels of plasma binding against Omicron Spike in previously infected individuals were similar to those against Delta Spike in naive individuals (Figure 1 I and 1J). In agreement with previous observations (Tauzin et al., 2022), Spike recognition declined more rapidly in the naive group compared with previously infected individuals.

Impact of the interval between mRNA vaccine doses on Omicron Spike recognition and neutralization

Recent reports suggested that vaccine regimens with a delayed boost elicit stronger humoral responses than the approved, short interval, regimen (Grunau et al., 2021; Tauzin et al., 2022). The long regimen interval has been associated with good vaccine ef-

ficacy against different VOCs (Skowronski et al., 2021). We therefore compared the capacity of plasma from naive vaccinated individuals who received the second dose with an interval of 16 weeks (median [range]: 111 days [76–120 days]) with those obtained from 19 SARS-CoV-2 naive donors who received their two doses 4 weeks apart (median [range]: 29 days [22–34 days]) (Table 1 and Figure 2A). As shown in Figure 2B, plasma from naive individuals who received a 16-week interval between the two doses recognized significantly better all tested Spikes, including Omicron, than plasma from individuals who received a short interval between doses (4 weeks). The 16-week interval regimen elicited significantly better neutralization activity against pseudoviral particles bearing the D614G, Beta, Delta, and Omicron Spikes (Figure 2C). Strikingly, this increased neutralization was more pronounced for the Omicron Spike (8.9-fold increase) compared with the other emerging variant Spikes (D614G, Beta, and Delta) (2.2- to 4.2-fold increase). This suggests that the delayed boosting in naive individuals facilitates antibody maturation resulting in enhanced breadth able to provide detectable levels of recognition and neutralization against Omicron.

DISCUSSION

In the province of Québec, Canada, like in other jurisdictions worldwide, the prevalence of Omicron increased dramatically from the first case detected on November 23 to being the dominant variant less than 1 month later. Compared with the reference Wuhan-Hu-1 strain, the Omicron variant carries more than 50 non-synonymous mutations within its genome, more than 30 of which are located in the gene coding for the Spike glycoprotein. Several of these mutations affecting the RBD, the N-terminal domain (NTD), and the furin cleavage domain were observed in other VOCs (Viana et al., 2021), which is consistent with positive selection of favorable mutations. Previous in vitro studies already showed the association of some of these mutations with increased infectivity, ACE2 interaction (N501Y, P681H) (Gong et al., 2021; Saito et al., 2021), or immune evasion (K417N, N440K, G446S, S477N, E484A/K, Q493R) (Baum et al., 2020; Clark et al., 2021; Greaney et al., 2021a, 2021b, 2021c; Liu et al., 2020; Rappazzo et al., 2021; Starr et al., 2021; Weisblum et al., 2020). This unprecedented accumulation of Spike mutations raised concern about a major antigenic drift that could significantly decrease the efficacy of current vaccines (Andrews et al., 2021; Khoury et al., 2021; Schmidt et al., 2021b).

To get a better understanding of the antigenic profile, we compared the antigenicity of the Omicron Spike with those from D614G, Alpha, Beta, Gamma, and Delta VOCs. We used

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Figure 1. Binding of vaccine-elicited antibodies to SARS-CoV-2 Spike variants

(A) SARS-CoV-2 vaccine cohort design.

(B–G) 293T cells were transfected with the indicated full-length Spike from different SARS-CoV-2 variants (D614G in B, Alpha in C, Beta in D, Gamma in E, Delta in F, and Omicron in G) and stained with the CV3-25 Ab or with plasma collected 3 weeks (V3) or 4 months (V4) after a second dose administered with a 16-week interval. Samples were analyzed by flow cytometry. The values represent the median fluorescence intensities (MFIs) normalized by CV3-25 Ab binding and presented as percentages of CV3-25 binding (B–G, left panels). Each curve represents the normalized MFIs obtained with the plasma of one donor at every time point. The mean of each group is represented by a bold line. In the right panels, plasma samples were grouped in different time points (V0, V3, and V4).

(H–J) Comparison of Spike recognition by plasma from naive and previously infected donors, represented by red and black points, respectively. Each symbol/points identifies one donor. Error bars indicate means ± SEM. For naive donors, n = 20 at V3 and V4. For previously infected donors vaccinated with two doses, n = 15 at V0 (H), V3 (I), and V4 (J). Statistical significance was tested using (B–G, left panels, (H, I, J) a Wilcoxon test, or (B–G, right panels) a Mann-Whitney test (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; ns, non-significant).

Table 1. Characteristics of the vaccinated SARS-CoV-2 cohorts

	SARS-CoV-2 naive		SARS-CoV-2 previously infected
	Two doses Short interval (n = 19)	Two doses Long interval (n = 25)	Two doses Long interval (n = 15)
Age	39 (20–74)	50 (21–62)	47 (29–65)
Sex			
Male (n)	12	11	10
Female (n)	7	14	5
Days between symptom onset and V0[a]	N/A	N/A	191 (85–234)
Days between symptom onset and the 1st dose[a]	N/A	N/A	274 (166–321)
Days between the 1st and 2nd dose[a]	29 (22–34)	111 (76–120)	110 (90–134)
Days between the 2nd dose and V3[a]	22 (12–53)	21 (14–34)	22 (13–51)
Days between the 2nd dose and V4[a]	N/A	112 (103–125)	113 (90–127)

[a]Values displayed are medians, with ranges in parentheses.

plasma from naive and previously infected individuals who received their two doses of the BNT162b2 mRNA vaccine 16 weeks apart. In agreement with previous observations, we found that previously infected vaccinated individuals recognized more efficiently all Spikes than naive individuals at the two timepoints analyzed (3 weeks and 4 months post second dose) (Stamatatos et al., 2021; Tauzin et al., 2021, 2022). Interestingly, we observed that recognition of all Spikes, including Omicron, decreased more rapidly in naive than previously infected individuals, as reported (Tauzin et al., 2022). The three antigenic exposures (infection +2 doses) of previously infected individuals compared with the two exposures in double-vaccinated naive individuals possibly explains their more sustained humoral response, suggesting that an additional exposition to the Spike antigen in the form of a third vaccine dose could elicit similar responses. Alternatively, if infection elicits a qualitatively broader humoral response linked to epitopes located outside the Spike glycoprotein, the effect of a third dose in naive individuals may remain qualitatively different. Independently of their infection history, all plasma recognized significantly less efficiently the Omicron Spike compared with Spikes from other VOCs (Figure 1). Of note, the sequence initially released for the Omicron Spike contained the Q493K substitution, but was then corrected to Q493R. Since initial studies on Omicron used the Q493K mutation, we verified whether the nature of the residue at 493 (either K or R) impacted plasma recognition or neutralization. We observed no significant differences among them (Figure S2). As indicated in the STAR methods section, all results generated in the current manuscript were done using the Q493R mutation.

Since recent studies have shown that recognition of full-length Spikes at the surface of transfected 293T cells strongly correlates with recognition of primary airway epithelial cells infected with authentic viruses as well as with antibody-dependent cellular cytotoxicity (Ding et al., 2022), these results suggest that Fc-mediated effector functions against Omicron could also be affected. Of note, low Spike recognition translated into increased Omicron neutralization resistance (Figure 2C). In agreement with previous observations (Stamatatos et al., 2021; Tauzin et al., 2021, 2022), plasma from vaccinated previously infected individuals recognized more efficiently Omicron and all other VOCs than vaccinated naive individuals (Figure 1). As naive double-vaccinated individuals have been well protected against the Delta variant, the observation of similar levels of plasma binding against Delta Spike in naive individuals and those against Omicron Spike in previously infected individuals may be important. This suggests that the benefits of hybrid immunity also apply to Omicron but this hypothesis will need confirmation through vaccine effectiveness studies.

Several reports (Cele et al., 2021; Garcia-Beltran et al., 2021; Planas et al., 2021a; Schmidt et al., 2021a; Zhang et al., 2021) have shown neutralization resistance using plasma from naive donors who received the approved regimen of the BNT162b2 mRNA vaccine (3- to 4-week vaccine interval). Strikingly, we observed that plasma from naive vaccinated donors who received their two doses according to the approved short 3- to 4-week interval, recognized and neutralized Omicron significantly less efficiently compared with the long 16-week interval. For all individuals, the level of Omicron Spike recognition remains lower than for the ancestral Spike, the antigen used in the current vaccines, and these levels decrease over time. Therefore, it will be important to determine in epidemiological studies if the vaccine interval advantage, as measured by these *in vitro* parameters, confers any clinical benefit against Omicron.

Limitations of the study

A limitation of our study is the relatively low number of individuals analyzed; however, we note that our results are in agreement with recent findings indicating that longer mRNA vaccine dosing intervals have improved immunogenicity (Grunau et al., 2021; Tauzin et al., 2022), which may have been associated with an optimized booster dose protection in Canada (Skowronski et al., 2021). Epidemiological studies will be required to establish the vaccine efficacy of the extended interval dosing against severe outcomes caused by Omicron.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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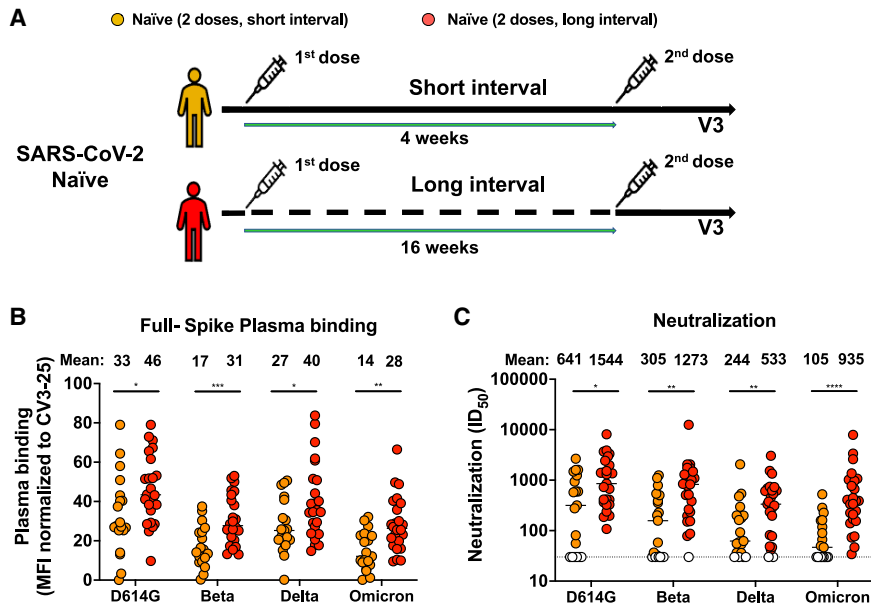 - Ethics statement

Figure 2. Omicron Spike recognition and neutralization with plasma from naive individuals who received a short versus a long mRNA vaccine dose interval

(A) SARS-CoV-2 vaccine cohort design.

(B) 293T cells were transfected with the full-length Spike from different SARS-CoV-2 variants (D614G, Beta, Delta, and Omicron) and stained with the CV3-25 Ab or with plasma from naive donors who received a short (4 weeks, yellow) or long (16 weeks, red) interval between doses collected 3 weeks after the second dose (V3) and analyzed by flow cytometry. The values represent the MFI normalized by CV3-25 Ab binding and presented as percentages of CV3-25 binding.

(C) Neutralizing activity was measured by incubating pseudoviruses bearing indicated SARS-CoV-2 Spikes (D614G, Beta, Delta, and Omicron), with serial dilutions of plasma for 1 h at 37°C before infecting 293T-ACE2 cells. Neutralization half-maximal inhibitory serum dilution (ID_{50}) values were determined using a normalized nonlinear regression using GraphPad Prism software. Undetectable measures are represented as white symbols, and limits of detection are plotted. Error bars indicate means ± SEM. For naive donors vaccinated with the short interval, n = 19. For naive donors vaccinated with the long interval, n = 25. Each symbol/points identifies one donor. Statistical significance was tested using a Mann-Whitney test (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).

- ○ Human subjects
- ○ Plasma and antibodies
- ○ Cell lines
- ● METHOD DETAILS
  - ○ Plasmids
  - ○ Cell surface staining and flow cytometry analysis
  - ○ Virus neutralization assay
- ● QUANTIFICATION AND STATISTICAL ANALYSIS
  - ○ Statistical analysis

AUTHOR CONTRIBUTIONS

D.C., J.R., S.M., M.C., and A.F. conceived the study. D.C., A.T., J.R., M.C., and A.F. designed experimental approaches. D.C., A.T., L.M., S.Y.G., M.B., C.B., G.B.-B., Y.B., S.D., A.L., D.V., E.F., A.G., B.D., I.L., G.G., G.G.-L., H.M., J.Prévost, J.R., M.C., and A.F. performed, analyzed, and interpreted the experiments. J.Perreault, L.G., C.M., P.A., C.T., V.M.-L., D.E.K., and R.B. collected and provided clinical samples. G.D.S., C.T., V.M.-L., and D.E.K. provided scientific input related to VOCs and vaccine efficacy. D.C., J.R., and A.F. wrote the manuscript with inputs from others. Every author has read, edited, and approved the final manuscript.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
LIVE-DEAD Fixable AquaVivid Cell Stain	Thermo Fischer Scientific	Cat# P34957
CV3-25	(Jennewein et al., 2021)	N/A
Alexa Fluor 647 AffiniPure Goat Anti-Human IgA + IgG + IgM (H+L)	Jackson ImmunoResearch	Cat # 109-605-064; RRID: AB_2337886
Goat anti-Human IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	Invitrogen	Cat # A-21445; RRID: AB_2535862
Biological samples		
SARS-CoV-2 naïve donor blood samples	This paper	N/A
SARS-CoV-2 previously infected donor blood samples	This paper	N/A
Chemicals, peptides, and recombinant proteins		
Dulbecco's Modified Eagle's medium (DMEM)	Wisent	Cat# 319-005-CL
Penicillin/Streptomycin	Wisent	Cat# 450-201-EL
Fetal Bovine Serum (FBS)	VWR	Cat# 97068-085
Phosphate Buffered Saline (PBS)	ThermoFischer Scientific	Cat# 10010023
Puromycin Dihydrochloride	Millipore Sigma	Cat# P8833
Passive lysis buffer	Promega	Cat # E1941
D-Luciferin Potassium Salt	Prolume	Cat # 306
Formaldehyde 37%	Thermo Fischer Scientific	Cat# F79-500
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	Cat # D2650-5X5ML CAS: 67-68-5
Experimental models: cell lines		
HEK293T cells	ATCC	Cat# CRL-3216; RRID: CVCL_0063
293T-ACE2 cells	(Prevost et al., 2020)	N/A
Recombinant DNA		
pNL4.3 R-E− Luc	NIH AIDS reagent program	Cat# 3418
pIRES2-EGFP	Clontech	Cat# 6029-1
pCG1-SARS-CoV-2 D614G-Spike	(Beaudoin-Bussieres et al., 2020)	N/A
pCDNA3.1-SARS-CoV-2-B.1.1.7 Spike	(Tauzin et al., 2021)	N/A
pcDNA3.1-SARS-CoV-2-B.1.351 Spike	(Gong et al., 2021)	N/A
pCAGGS-SARS-CoV-2-B.1.617.2 Spike	(Gong et al., 2021)	N/A
pcDNA3.1-SARS-CoV-2-P.1 Spike	(Gong et al., 2021)	N/A
pCAGGS-SARS-CoV-2 B.1.1.529 Spike	This paper	N/A
Software and algorithms		
Flow Jo v10.7.1	Flow Jo	https://www.flowjo.com
GraphPad Prism v8.4.3	GraphPad	https://www.graphpad.com
Microsoft Excel v16	Microsoft Office	https://www.microsoft.com/en-ca/microsoft-365/excel
Others		
BD LSRII Flow Cytometer	BD Biosciences	N/A
TriStar LB942 Microplate Reader	Berthold Technologies	N/A
Nanodrop Spectrophotometer ND-1000	ThermoFisher Scientific	N/A

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Andrés Finzi (andres.finzi@umontreal.ca).

Materials availability
All unique reagents generated during this study are available from the lead contact without restriction.

Data and code availability
- All data reported in this paper will be shared by the lead contact (andres.finzi@umontreal.ca) upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact (andres.finzi@umontreal.ca) upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Ethics statement
All work was conducted in accordance with the Declaration of Helsinki in terms of informed consent and approval by an appropriate institutional board. Blood samples were obtained from donors who consented to participate in this research project at CHUM (19.381) and from plasma donors who consented to participate in the Plasma Donor Biobank at Hema-Quebec (PLASCOV; REB-B-6-002-2021-003). Plasma was isolated by centrifugation, and samples stored at -80°C and in liquid nitrogen, respectively, until use.

Human subjects
The study was conducted in 25 SARS-CoV-2 naïve individuals (11 males and 14 females; age range: 21–62 years) vaccinated with a long interval, 19 SARS-CoV-2 naïve individuals (12 males and 7 females; age range: 20–74 years) vaccinated with a short interval and 15 SARS-CoV-2 previously-infected individuals (10 males and 5 females; age range: 29–65 years) vaccinated with a long interval. All this information is summarized in Table 1. No specific criteria such as number of patients (sample size), gender, clinical or demographic were used for inclusion, beyond PCR confirmed SARS-CoV-2 infection in adults.

Plasma and antibodies
Plasma from SARS-CoV-2 naïve and previously-infected donors were collected, heat-inactivated for 1 hour at 56°C and stored at -80°C until ready to use in subsequent experiments. The conformationally independent S2-specific monoclonal antibody CV3-25 (Gong et al., 2021; Jennewein et al., 2021; Li et al., 2021; Prevost et al., 2021; Ullah et al., 2021) was used as a positive control and to normalize Spike expression in our flow cytometry assays, as described (Gong et al., 2021; Tauzin et al., 2021, 2022). Alexa Fluor-647-conjugated goat anti-human Abs (Invitrogen) were used as secondary antibodies to detect plasma binding in flow cytometry experiments.

Cell lines
293T human embryonic kidney cells (obtained from ATCC) were maintained at 37°C under 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) (Wisent) containing 5% fetal bovine serum (FBS) (VWR) and 100 μg/mL of penicillin-streptomycin (Wisent). The 293T-ACE2 cell line was previously reported (Prevost et al., 2020).

METHOD DETAILS

Plasmids
The plasmids encoding the SARS-CoV-2 Spike variants; D614G, B.1.1.7, B.1.351, P.1 and B.1.617.2 were previously described (Beaudoin-Bussieres et al., 2020; Gong et al., 2021; Li et al., 2021; Tauzin et al., 2021, 2022). The plasmids encoding the B.1.1.529 Spike was generated by overlapping PCR using a codon-optimized wild-type SARS-CoV-2 Spike gene (GeneArt, Thermo-Fisher) that was synthesized (Biobasic) and cloned in pCAGGS as a template. The B.1.1.529 Spike coding sequence was derived from the sequence ID EPI_ISL_6640919. This sequence initially contained the Q493K substitution, as previously reported (Cameroni et al., 2021; Schmidt et al., 2021a; Shah and Woo, 2021). The ECDC (European Centre for Disease Prevention and Control) later informed that Omicron spike actually have an R mutation at position 493. We therefore generated and used an Omicron Spike bearing the Q493R mutation for the full manuscript (Figures 1 and 2 and Figure S1). Nevertheless, we compared whether the nature of the residue (either K or R) at this position impacted plasma recognition and/or neutralization activity; no significant differences were observed (Figure S2).

Cell surface staining and flow cytometry analysis

293T were transfected with full-length SARS-CoV-2 Spikes and a green fluorescent protein (GFP) expressor (pIRES2-eGFP; Clontech) using the calcium-phosphate method. Two days post-transfection, Spike-expressing 293T cells were stained with the CV3-25 Ab (5 μg/mL) as control or plasma from vaccinated individuals (1:250 dilution) for 45 min at 37°C. AlexaFluor-647-conjugated goat anti-human IgG (1/1000 dilution) were used as secondary Abs. The percentage of Spike-expressing cells (GFP + cells) was determined by gating the living cell population based on viability dye staining (Aqua Vivid, Invitrogen). Samples were acquired on a LSR II cytometer (BD Biosciences), and data analysis was performed using FlowJo v10.7.1 (Tree Star). The conformationally-independent anti-S2 antibody CV3-25 was used to normalize Spike expression, as reported (Gong et al., 2021; Li et al., 2021; Prevost et al., 2021; Ullah et al., 2021). CV3-25 was shown to be effective against all Spike variants (Gong et al., 2021; Li et al., 2021; Prevost et al., 2021; Ullah et al., 2021) and (Figure S1). The Median Fluorescence intensities (MFI) obtained with plasma were normalized to the MFI obtained with CV3-25 (Gong et al., 2021; Li et al., 2021; Prevost et al., 2021; Ullah et al., 2021) and presented as percentage of CV3-25 binding.

Virus neutralization assay

To produce SARS-CoV-2 pseudoviruses, 293T cells were transfected with the lentiviral vector pNL4.3 R-E– Luc (NIH AIDS Reagent Program) and a plasmid encoding for the indicated S glycoprotein (D614G, Alpha, Beta, Gamma, Delta or Omicron) at a ratio of 10:1. Two days post-transfection, cell supernatants were harvested and stored at −80°C until use. For the neutralization assay, 293T-ACE2 target cells were seeded at a density of 1×10^4 cells/well in 96-well luminometer-compatible tissue culture plates (PerkinElmer) 24h before infection. Pseudoviral particles were incubated with several plasma dilutions (1/50; 1/250; 1/1250; 1/6250; 1/31250) for 1h at 37°C and were then added to the target cells followed by incubation for 48h at 37°C. For CV3-25 neutralization, pseudoviral particles were incubated with increasing concentrations of CV3-25 (0.01, 0.0316, 0.1, 0.316, 1 and 3.16 μg/mL) for 1h at 37°C and were then added to the target cells followed by incubation for 48h at 37°C. Cells were lysed by the addition of 30 μL of passive lysis buffer (Promega) followed by one freeze-thaw cycle. An LB942 TriStar luminometer (Berthold Technologies) was used to measure the luciferase activity of each well after the addition of 100 μL of luciferin buffer (15mM $MgSO_4$, 15mM KH_2PO_4 [pH 7.8], 1mM ATP, and 1mM dithiothreitol) and 50 μL of 1mM d-luciferin potassium salt (Prolume). The neutralization half-maximal inhibitory dilution (ID_{50}) represents the plasma dilution to inhibit 50% of the infection of 293T-ACE2 cells by pseudoviruses.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis

Symbols represent biologically independent samples from SARS-CoV-2 naïve or PI individuals. Lines connect data from the same donor. Statistics were analyzed using GraphPad Prism version 8.0.1 (GraphPad, San Diego, CA). Every dataset was tested for statistical normality and this information was used to apply the appropriate (parametric or nonparametric) statistical test. p values < 0.05 were considered significant; significance values are indicated as *P <0.05, **P<0.01, ***P<0.001, **** P<0.0001, ns, non-significant.