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A single dose of the SARS-CoV-2 vaccine BNT162b2 elicits Fc-mediated antibody effector functions and T cell responses

Highlights

- Three weeks after the first BNT162b2 dose, weak neutralizing antibodies are elicited
- These antibodies have robust Fc-mediated effector functions
- Vaccination of individuals previously infected boosts humoral and cellular responses
- Strong correlations between T helper cell and humoral responses are observed

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In brief

Tauzin and Nayrac et al. characterize humoral and cellular responses 3 weeks after a single dose of mRNA BNT162b2 vaccine. They show, in SARS-CoV-2-naive individuals, that the antibodies elicited have weak neutralizing activity but potent Fc-mediated effector functions, and in SARS-CoV-2 previously infected individuals, that all responses are significantly boosted.

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A single dose of the SARS-CoV-2 vaccine BNT162b2 elicits Fc-mediated antibody effector functions and T cell responses

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SUMMARY
While the standard regimen of the BNT162b2 mRNA vaccine for SARS-CoV-2 includes two doses administered 3 weeks apart, some public health authorities are spacing these doses, raising concerns about efficacy. However, data indicate that a single dose can be up to 90% effective starting 14 days post-administration. To assess the mechanisms contributing to protection, we analyzed humoral and T cell responses three weeks after a single BNT162b2 dose. We observed weak neutralizing activity elicited in SARS-CoV-2 naive individuals but strong anti-receptor binding domain and spike antibodies with Fc-mediated effector functions and cellular CD4+ T cell responses. In previously infected individuals, a single dose boosted all humoral and T cell responses, with strong correlations between T helper and antibody immunity. Our results highlight the potential role of Fc-mediated effector functions and T cell responses in vaccine efficacy. They also provide support for spacing doses to vaccinate more individuals in conditions of vaccine scarcity.

INTRODUCTION
The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiological agent of the coronavirus disease 2019 (COVID-19), responsible for the current pandemic that infected over 174 million people and led to more than 3.74 million deaths worldwide (Dong et al., 2020; World Health Organization). This pandemic caused a race for the elaboration of an effective vaccine against SARS-CoV-2 (Krammer, 2020; Moore and Klasse, 2020). Currently approved vaccines target the highly immunogenic trimeric spike (S) glycoprotein that facilitates SARS-CoV-2 entry into host cells via its receptor-binding domain (RBD), which interacts with angiotensin-converting enzyme 2 (ACE-2) (Hoffmann et al., 2020; Walls et al., 2020). Among these vaccines, four are approved in many countries (Pfizer/BioNTech BNT162b2, Moderna mRNA-1273, AstraZeneca ChAdOx1, and Janssen Ad26.COV2S). The Pfizer/BioNTech BNT162b2 vaccine was developed using a novel technology based on mRNA (Polack et al., 2020). This technology consists of intramuscular injection of a lipid nanoparticle-encapsulated synthetic mRNA vaccine encoding the viral S glycoproteins of SARS-CoV-2, which has shown to elicit a robust efficacy against the Wuhan-Hu-1 strain, which served as template for their development (Baden et al., 2021; Sahin et al., 2020a). This vaccine encodes
for a membrane-anchored SARS-CoV-2 full-length S, stabilized in a prefusion conformation by mutating the furin cleavage site and introducing two prolines in the S2 fusion machinery (Polack et al., 2020; Wrapp et al., 2020). However, the emergence of mutations in the SARS-CoV-2 S glycoprotein could affect different properties of the virus including affinity for its receptor, resulting in increased infectivity, transmissibility, and evasion from humoral responses elicited by natural infection or vaccination (Prévost and Finzi, 2021).

The D614G S mutation appeared very early in the pandemic and is now highly prevalent in all circulating strains (Isabel et al., 2020). The B.1.1.7 variant was first identified in the United Kingdom and has been spread rapidly to many countries since its identification. This variant contains several mutations in its S glycoprotein (ΔH69-V70, ΔY144, N501Y, A570D, P681H, T716I, S982A, and D1118H) and has increased infectivity (Davies et al., 2020; Rambaut et al., 2020). Among the mutations present in the B.1.1.7 strain, the N501Y is also present in many other circulating variants (B.1.351 and P.1) and increases the affinity for the ACE-2 receptor (Chan et al., 2020b; Corum and Zimmer, 2021). The E484K mutation is part of the South African B.1.351 variant and is now found in several SARS-CoV-2 genomes worldwide that spread rapidly (Grots Ferrareze et al., 2021).

Studies have shown that this mutation increases affinity of the S glycoprotein for ACE-2 (Nelson et al., 2021) and confers resistance to neutralization mediated by monoclonal antibodies (mAbs) and plasma from naturally infected and vaccinated individuals (Ku et al., 2021; Stamatatos et al., 2021; Tada et al., 2021; Wang et al., 2021). The S477N mutation confers a higher affinity for the ACE-2 receptor and has rapidly spread to many countries in Oceania and Europe (Flores-Alanis et al., 2021; Hodcroft et al., 2020; Jolly et al., 2021; Mejdani et al., 2021; West et al., 2021; Wu et al., 2021). The S477N and N501S mutations are found in several SARS-CoV-2 genomes in Quebec (S.M. and M.R., unpublished data).

In spite of the proven clinical efficacy of BNT162b2, there are still limitations in the understanding of the protective components of the immune responses elicited by this vaccine. Such protection is mediated through a complex interplay between innate, humoral, and cell-mediated immunity (Pulendran and Ahmed, 2011; Sallusto et al., 2010). Several reports showed that administration of the mRNA vaccine induced a strong humoral response after two doses, especially against the RBD domain (Ju et al., 2020; Wu et al., 2020). Robust CD4+ and CD8+ memory T cell responses are induced after SARS-CoV-2 infection (Breton et al., 2021; Dan et al., 2021) and play important roles in resolution of the infection (Sette and Crotty, 2021) including modulating disease severity in humans (Rydzynski Moderbacher et al., 2020) and reducing viral load in non-human primates (NHPs) (Muñoz-Fontela et al., 2020). However, the detection of these specific memory T cells has been poorly studied in the SARS-CoV-2 vaccine development and represents a gap in the understanding of the induced cellular adaptive immune responses, which are likely to also play an important role (Sahin et al., 2020a). Among CD4+ T cells, the T follicular helper (Tfh) subset is of particular interest, as it provides help for B cell maturation and development of high-affinity antibody (Ab) responses in the germinal center (GC) of secondary lymphoid organs. Studies have shown that a subset of CXCR5+ in blood, called circulating Tfh (cTfh) (Crotty, 2014; Morita et al., 2011), has clonal, phenotypic, and functional overlap with GC Tfh and reflects, at least in part, responses in tissues (Heit et al., 2017; Vella et al., 2019).

Results from phase III clinical trials have shown a vaccine efficacy of >90% starting 14 days after the injection of a single dose of BNT162b2 mRNA vaccine, thus before the administration of a second dose (Baden et al., 2021; Polack et al., 2020; Skowronski et al., 2021). In this report, we characterized the humoral and T cell immune responses in cohorts of SARS-CoV-2 naive and naturally infected individuals prior and 3 weeks after a first dose of the BNT162b2 mRNA vaccine.

RESULTS

Here, we analyzed humoral and cellular responses in blood samples from 24 SARS-CoV-2 naive donors prior and after vaccination (median [range]: 21 days [16–28 days]). In addition, we examined the same immunological features in 24 individuals that were previously infected (PI) around 9 months before vaccination (median [range]: 279 days [61–326 days]) and 3 weeks after vaccination (median [range]: 21 days [13–26 days]). For 16 of these donors, we also longitudinally monitored evolution of the humoral response, from 6 weeks post-symptom onset (PSO, median [range]: 44 days [16–95 days]) to 3 weeks after vaccination. Basic demographic characteristics are summarized in Table 1. In the SARS-CoV-2 naive group, the average age of donors was 46 years (range: 21–59 years), and samples were from 7 males and 17 females. In the group of PI individuals, the average age of the donors was 47 years (range: 23–65 years), and samples were from 10 males and 14 females (Table 1).

Elicitation of SARS-CoV-2 antibodies against the full spike and its receptor-binding domain

To evaluate vaccine responses in SARS-CoV-2 naive and PI individuals, we first measured the presence of RBD-specific Abs (IgG, IgM, and IgA) using a previously described enzyme-linked immunosorbent (ELISA) RBD assay (Beaudoin-Bussières et al., 2020; Gasser et al., 2021; Prévost et al., 2020). As expected, in the SARS-CoV-2 naive group, we did not observe RBD-specific immunoglobulins (Ig) in samples recovered before vaccination.
Three weeks after the first dose, we found a significant increase in the total RBD-specific Ig levels with the exception of one donor from the naive group who didn’t respond to the vaccine at this time point. Vaccination induced similar levels of IgM and IgA targeting the RBD to those present in individuals that were naturally infected 9 months before (Figures 1B and 1C). In addition, RBD-specific IgG levels were significantly higher in the vaccinated SARS-CoV-2 naive group compared to pre-vaccination samples from the PI participants (Figure 1D).

In the group of individuals that were PI, despite a decline in the amount of anti-RBD-specific antibodies over time after infection (Figures S1A–S1D), most donors still had detectable anti-RBD-specific Abs just before vaccination, especially anti-RBD IgG (Figures 1A–1D). For all participants, the first dose of vaccination led to a robust increase in anti-RBD IgG and anti-RBD IgA levels, higher than the first time point measured PSO (16–95 days; median: 44 days) (Figures 1C–1D and S1C–S1D). Vaccination modestly increased the level of RBD-specific IgM (Figures 1B and S1B). Among the studied humoral responses, anti-SARS-CoV-2 neutralization returned to baseline most promptly, whereas antibody-dependent cellular cytotoxicity (ADCC) remained more robust in the convalescent stage while still responding with a significant increase post-vaccination (Figures S1E–S1G).

To evaluate whether vaccine responses were limited to RBD or could be extended to Abs against the full S glycoprotein, we used a cell-based ELISA (CBE) assay to measure levels of Abs recognizing the native full-length SARS-CoV-2 S. Anti-S Ab binding was detected using HRP-conjugated (E) anti-human IgM+IgG+IgA, (F) anti-human IgM, (G) anti-human IgA, or (H) anti-human IgG. RLU values obtained with parental HOS (negative control) were subtracted and further normalized to the signal obtained with the anti-RBD CR3022 mAb present in each plate.

**Figure 1. Elicitation of RBD- and spike-specific antibodies by a single dose of Pfizer/BioNTech mRNA vaccine in SARS-CoV-2 naive and previously infected individuals**

(A–D) Indirect ELISA was performed by incubating plasma samples from naive and PI donors collected before and after the first dose of vaccine with recombinant SARS-CoV-2 RBD protein. Anti-RBD Ab binding was detected using HRP-conjugated (A) anti-human IgM+IgG+IgA, (B) anti-human IgM, (C) anti-human IgA, or (D) anti-human IgG. Relative light unit (RLU) values obtained with BSA (negative control) were subtracted and further normalized to the signal obtained with the anti-RBD CR3022 mAb present in each plate.

(E–H) Cell-based ELISA was performed by incubating plasma samples from naive and PI donors collected before and after the first dose of vaccination with HOS cells expressing full-length SARS-CoV-2 S. Anti-S Ab binding was detected using HRP-conjugated (E) anti-human IgM+IgG+IgA, (F) anti-human IgM, (G) anti-human IgA, or (H) anti-human IgG. RLU values obtained with parental HOS (negative control) were subtracted and further normalized to the signal obtained with the CR3022 mAb present in each plate.

Limits of detection are plotted (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; ns, non-significant).
The individual who did not elicit anti-RBD Abs upon vaccination didn’t elicit Abs against other regions of the S, with detection levels no higher than our seropositivity threshold level (Figures 1E–1H).

Thus, vaccination in the SARS-CoV-2 naive group elicited Abs against the RBD and full S that reached similar levels than in naturally infected individuals 9 months PSO (Figure 1).

Recognition of SARS-CoV-2 spike variants and other Betacoronaviruses

SARS-CoV-2 is evolving, and variants of concern are emerging globally. Some harbor specific mutations in S that are associated with increased transmissibility and/or immune evasion (Davies et al., 2020; Sabino et al., 2021; Tegally et al., 2020; Volz et al., 2021). To evaluate whether a single dose of the Pfizer/BioNTech vaccine elicits Abs that are able to recognize a broader spectrum of variants, including S with putative escape mutations, we measured the ability of plasma from vaccinated individuals to recognize different S variants expressed at the cell surface by flow cytometry, using a method we recently reported (Prévost et al., 2020). As expected, none of the SARS-CoV-2 naive plasma samples obtained before vaccination (baseline) recognized the SARS-CoV-2 S (D614G) or any of its variants (Figures 2A–2E). However, they were able to recognize S from endemic human coronaviruses (HCoV-OC43 and HCoV-HKU1) but not S from highly pathogenic coronaviruses (SARS-CoV-1 and MERS-CoV) (Figures 2F–2I). In agreement with our CBE results (Figure 1), vaccination elicited Abs that efficiently recognized the full S and all the tested variants (Figures 2A–2E), except for the same donor that did not elicit RBD- or S-specific Abs. The recognition levels were equivalent to those observed for PI individuals before vaccination. In the latter group, all plasma samples recognized the different S variants before vaccination, and the first dose of vaccine led to a significant increase in S recognition (Figures 2A–2E). When we compared the differences in recognition between the SARS-CoV-2 variants, we observed that plasma from vaccinated naive individuals recognized the different SARS-CoV-2 variants less efficiently compared to D614G S (Figure S2A). Plasma from PI individuals recognized all SARS-CoV-2 S before and after vaccination (Figures S2B and S2C). Vaccination, however, robustly enhanced recognition in this group, albeit a bit less efficiently for the S variants (Figure S2C).

We recently reported that SARS-CoV-2 infection elicits cross-reactive Abs that can recognize S from other human coronaviruses (Beaudoin-Bussières et al., 2020; Prévost et al., 2020). To evaluate whether vaccination also elicited Abs able to recognize S glycoproteins from other Betacoronaviruses, we evaluated the capacity of the different plasma samples to bind cell-surface-expressed S from SARS-CoV-1, MERS-CoV, HCoV-OC43, and HCoV-HKU1. As shown in Figure 2 (2F–2I), vaccination elicited cross-reactive Abs in both groups with enhanced recognition of SARS-CoV-1, MERS-CoV, HCoV-HKU1, and HCoV-OC43 only in the SARS-CoV-2 naive group.

Functional activities of vaccine-elicited antibodies

A single dose of the Pfizer/BioNTech vaccine was shown to be up to 90% efficacious starting 2 weeks after administration (Skowronski and De Serres, 2021; Skowronski et al., 2021; Polack et al., 2020). Among the immune responses elicited by the different SARS-CoV-2 vaccines, the neutralizing response is thought to be associated with vaccine efficacy (Jackson et al., 2020; Muruato et al., 2020; Polack et al., 2020). To evaluate whether neutralizing responses were elicited within the first 3 weeks upon vaccine administration, we measured the capacity of plasma samples to neutralize pseudoviral particles carrying the SARS-CoV-2 S glycoprotein. All pseudovirus variants were infectious in this system with SARS-CoV-2 variants, with B.1.1.7 in particular exhibiting enhanced infectivity (Figure S2D).

We observed very low neutralizing activity in plasma from vaccinated naive individuals (Figure 3A), in agreement with previous findings (Collier et al., 2021; Ellebedy et al., 2021; Goel et al., 2021; Planas et al., 2021; Sahin et al., 2020b). As recently described (Krammer et al., 2021; Stamatakis et al., 2021), we observed that pre-existing SARS-CoV-2 neutralizing Ab responses were significantly boosted by a single dose of S-encoded mRNA vaccine (Figure 3A). Interestingly, a single dose enlarged the potency of the neutralizing response that was now able to efficiently neutralize pseudoviral particles bearing the B.1.1.7 S or from other variants with different concerning mutations (E484K, S477N, N501Y, and N501S) (Figures S2E–S2G). Remarkably it also boosted neutralization activity against pseudoparticles bearing the SARS-CoV-1 S (Figure S2H).

Since weak neutralizing activity was detected in SARS-CoV-2 naive vaccinated individuals, we decided to measure Fc-mediated effector functions that were also shown to play an important role against SARS-CoV-2 infection (Chan et al., 2020a; Schäfer et al., 2021; Zohar et al., 2020). In agreement with the lack of S-specific Abs, SARS-CoV-2 naive individuals did not have detectable ADCC activity prior to vaccination (Figure 3B). The first dose of the vaccine induced a significant increase in ADCC activity, except for one sample, corresponding to the donor who had not developed anti-S Abs. We noted that ADCC activity in vaccinated naive individuals achieved comparable levels to those of PI individuals before vaccination. Vaccination of this group significantly boosted the ADCC activity (Figure 3B). Based on these results, it is tempting to speculate that the generation of Abs with Fc-mediated effector functions, but with low neutralizing activity, might be sufficient to provide a certain level of protection.

Spike-specific T cell vaccine responses differ between SARS-CoV-2 naive and previously infected individuals

We examined whether prior SARS-CoV-2 infection alters the CD4+ and CD8+ T cell responses to vaccination. To measure SARS-CoV-2-S-specific T cells in the two cohorts of naive persons and PI individuals, we utilized two complementary methodologies, T cell receptor (TCR)-dependent activation-induced marker (AIM) assays and intracellular cytokine staining (ICS).
We performed the cytokine-independent AIM assays as previously described (Reiss et al., 2017), with some modifications. We stimulated PBMC for 15 h with an overlapping peptide pool spanning the S coding sequence and measured upregulation of the markers CD69, CD40L, 4-1BB, and OX-40 upon stimulation. We used an AND/OR Boolean combination gating strategy to identify antigen-specific T cell responses (Figure S3A) (Niessl et al., 2020a). We examined three populations of SARS-CoV-2-S-specific AIM+ T cells: (1) AIM+ total CD4+ T cells (Figure 4A), (2) AIM+ cTfh cells (Figure 4B), and (3) AIM+ total CD8+ T cells (Figure 4C). We and others have shown that AIM assays can sensitively detect infection- and vaccine-induced cTfh responses (Dan et al., 2016; Niessl et al., 2020b), including in naive individuals, dominant S-specific CD4+ T subsets that are preferentially increased by vaccination in PI included S-specific cTfh cells, independently of prior SARS-CoV-2 infection status, immunization elicited more robust and functionally skewed responses in participants with a history of SARS-CoV-2 infection, compared to naive people, with preferential expansion of specific functional subsets.

To qualitatively assess S-specific T cells in naive and PI groups for polyfunctional responses after vaccination, we performed co-expression analysis using Boolean gating and examined each combination of function (Figures 4F and 4G). In comparison to naive individuals, dominant S-specific CD4+ T subsets that were preferentially increased by vaccination in PI included S-specific CD4+ T cells coexpressing CD40L, or IFN-γ alone or in combination with other functions (TNF-α, CD107a, IL-2). The frequency of S-specific CD8+ T cells expressing IFN-γ alone or combined with CD107a was also increased in PI compared to naive participants.

These data show that while a single dose of mRNA vaccine could induce detectable S-specific CD4+ and CD8+ T cells in most individuals, including S-specific cTfh cells, independently of prior SARS-CoV-2 infection status, immunization elicited more robust and functionally skewed responses in participants with a history of SARS-CoV-2 infection, compared to naive people, with preferential expansion of specific functional subsets.

**Relationship between SARS-CoV-2-spike-specific T cell responses and humoral responses**

Most protective Ab responses are dependent on CD4+ T cell help, which is critical for B cell expansion, affinity maturation, and isotype switching. Therefore, we assessed whether pre-existing SARS-CoV-2-S-specific CD4+ T cells and cTfh responses were predictive of higher Ab titers and Ab functions, as measured by neutralization capacity and ADCC after vaccination, irrespective of prior infection status (Figure S3A). We found that correlations between the function-agnostic AIM+ CD4+

(A) Neutralizing activity was measured by incubating pseudoviruses bearing SARS-CoV-2 S glycoproteins, with serial dilutions of plasma for 1 h at 37°C before infecting 293T-ACE2 cells. Neutralization half maximal inhibitory serum dilution (ID50) values were determined using a normalized non-linear regression using GraphPad Prism software. (B) CEM.NKr parental cells were mixed at a 1:1 ratio with CEM.NKr-S cells and were used as target cells. PBMCs from uninfected donors were used as effector cells in a FACS-based ADCC assay. Limits of detection are plotted (**p < 0.01; ***p < 0.001; ns, non-significant).
Figure 4. Spike-specific CD4+ and CD8+ T cell vaccine responses quantitatively and qualitatively differ in SARS-CoV-2 naive versus previously infected individuals.

(A–C) Net frequencies after S peptide pool stimulation of (A) total S-specific AIM+ CD4+ T cells, (B) S-specific AIM+ cTfh, (C) S-specific AIM+ CD8+ T cells in each donor prior to (V0) and post- (V1) vaccination in the SARS-CoV-2 naive participants and those with previous SARS-CoV-2 infection.

(D–F) Representative absolute frequencies of (D) ICS CD4+ T cells and (E) ICS CD8+ T cells in each donor prior to (V0) and post- (V1) vaccination in the SARS-CoV-2 naive participants and those with previous SARS-CoV-2 infection.

(legend continued on next page)
T cell measurements and Ab responses were generally stronger than between ICS + CD4+ T cell responses and serological measurements (Figure 5A). Notably, the Ig subsets measured after vaccination in the plasma of each participant showed significant positive correlations between pre-existing S-specific CD4+ T cell and cTfh responses on the one hand, and anti-S IgA and IgG post-vaccination on the other hand (Figure 5C, 5D, 5F, and 5G). In contrast, we observed no significant correlations between total S-specific CD4+ T cell responses and anti-S IgM levels (Figure 5B) and between S-specific cTfh responses and anti-S IgM levels (Figure 5E). At the functional level, we observed significant correlations between all the pre-vaccination AIM+ S-specific memory CD4+ T cells and cTfh with ADCC and neutralization capacity post-immunization (Figure 5A).

These results suggest that pre-existing CD4+ T cell responses are beneficial for the generation of specific and effective humoral responses against SARS-CoV-2 after a single dose of mRNA vaccine, independently of prior SARS-CoV-2 infection.

Evaluation of vaccine responses

Assessing the humoral responses revealed that the vaccine-induced responses in naive individuals show striking similarities with the induced responses upon natural infection. With a few exceptions such as the neutralizing Ab response, at least for the given time points, the induced responses are similar (Figures 1, 2, and 3). This translates into a similar network of pairwise correlations among all studied parameters when comparing discrete time points before vaccination in infected individuals...
and post-vaccination in naive individuals (Figure 6). As expected, naive individuals before vaccination harbor hardly any interrelations between humoral and cellular anti-SARS-CoV-2 responses, which is in line with their overall low and unspecific absolute levels. Notably, when studying the effects of vaccination in PI individuals, the pairwise correlations are not getting stronger among our studied parameters, but the network of significant associations is broadened involving more interconnected parameters. It indicates that a heterogeneous boost, in this case an S mRNA vaccination boost upon natural infection as prime, brings in new flavors of host responses while diluting others.

To investigate whether pre-existing humoral responses before vaccination predict the levels of induced/boosted responses upon vaccination, we performed a tandem correlation analysis focusing on pairs of correlations between time points before versus after vaccination (Figure S4). In naive individuals, as expected, the low and SARS-CoV-2-unspecific responses before vaccination didn’t predict responses induced by vaccination. In contrast, individuals with previous SARS-CoV-2 infection harbor a much broader set of parameters pre-vaccination that predict induced responses post-vaccination in the studied dataset. Of interest, these correlations differ from the few observed in naive individuals. In PI individuals, most prominent patterns include the predictive value of binding, ADCC, and neutralization responses pre-vaccination for IgA responses in CBE assays and neutralization against viruses with the E484K S escape mutation post-vaccination.

DISCUSSION

The mRNA vaccines have demonstrated a >90% efficacy starting 14 days after a single dose, but the immune correlate of protection after a single dose remains unknown (Baden et al., 2021; Polack et al., 2020; Skowronski et al., 2021). Here, we measured several serological and cellular SARS-CoV-2-specific responses in SARS-CoV-2 naive or PI individuals. Surprisingly, despite the proven vaccine efficacy 3 weeks after vaccination (Baden et al., 2021; Polack et al., 2020; Skowronski et al., 2021), we observed weak neutralizing activity in plasma from SARS-CoV-2 naive vaccinated individuals, in agreement with recent studies (Collier et al., 2021; Ellebedy et al., 2021; Goel et al., 2021; Planas et al., 2021; Sahin et al., 2020b). Neutralization is thought to play a
central role in SARS-CoV-2 vaccine efficacy (Jackson et al., 2020; Muruato et al., 2020; Polack et al., 2020); however, recent observations suggest that they might not be predictive, on their own, of protection (Emary et al., 2021; Luchsinger and Hillyer, 2021). Affinity maturation through GC selection can lead to more potent neutralizing Ab responses. While kinetics may differ according to the antigen used and route of administration, measurable neutralizing titers may take several weeks to develop in humans and NHPs after immunization (Cirelli et al., 2019), and even after neutralization titers begin to decrease, the somatic hypermutation (SHM) process can continue for months after acute SARS-CoV-2 infection (Gaebler et al., 2021). Our results suggest that while the neutralization potency of vaccine-elicted Abs is being developed, other Ab functions such as Fc-mediated effector functions could contribute to vaccine efficacy early on. Accordingly, 3 weeks after a single dose, we observed strong ADCC but weak neutralization activity (Figure 3). Vaccination induced strong levels of IgG in both groups, thus it would be interesting to study which subclasses of IgG were induced and if it correlates with the effector functions observed. Strikingly, vaccination of PI individuals induced a very significant increase of pre-existing humoral immunity including ADCC and neutralization. These results are consistent with several recent studies which have shown that a single vaccine dose in PI individuals led to similar humoral responses compared with two vaccine doses in naive individuals, and they suggest that the second dose may not be necessary for PI individuals (Ebiner et al., 2021; Krammer et al., 2021; Saadat et al., 2021). Neutralization potency was increased, enabling neutralization of several variants including the B.1.1.7 variant, S with the E484K mutation, and even the phylogenetically more distant SARS-CoV-1.

We also demonstrated that the patterns of vaccine-induced T cell responses have analogies with those observed for Ab immunity. A single dose of BNT162b2 mRNA vaccine is also capable of generating SARS-CoV-2-specific T cell immune responses in both groups of individuals with a dominant CD4+ T cell response, suggesting efficacy of the priming immunization in generating cellular immunity against SARS-CoV-2 S. However, we observed differences in the magnitude and quality of these responses between participants with and without prior infection. Individuals who had already encountered SARS-CoV-2 developed strong S-specific memory CD4+ and CD8+ T cells, consistent with secondary memory responses to a recall antigen. In contrast, naive individuals showed significantly weaker S-specific CD4+ T cell responses and low to undetectable S-specific CD8+ T cell responses by AIM and ICS. Even though pre-vaccination T cell responses to SARS-CoV-2 S glycoprotein were minimal in most naive participants, it is still possible that the vaccine amplifies pre-existing CD4+ cross-reactive T cell responses to endemic human coronaviruses. This suggests that a single dose of mRNA vaccine may be sufficient to elicit robust protective T cell responses in PI individuals; naive persons will likely benefit most from repeat immunization.

Our results support the parallel use of both AIM and ICS assays for SARS-CoV-2 vaccine immunomonitoring. While most clinical trials relied on the IFN-γ ELISPOT assay and/or ICS to measure T cell responses, our data suggest the notion that BNT162b2 and some other SARS-CoV-2 vaccines in advanced clinical evaluation, including non-mRNA-vaccines, preferentially elicit Th1 responses and may have to be reconsidered (Corbett et al., 2020; van Doremalen et al., 2020; Ewer et al., 2021; Laczkó et al., 2020; Zhang et al., 2020). Indeed, these assays are sensitive for detection of Th1 cytokines and cytotoxic responses, but largely miss other important components of virus-specific cellular immunity. Consistent with this, we found that AIM assays were highly sensitive to detect vaccine-induced CD4+ and CD8+ T cell responses, consistent with recent findings in natural SARS-CoV-2 infection and vaccination (Dan et al., 2021; Kalimuddin et al., 2021). Still, ICS assays were essential to reveal qualitative differences in cellular responses elicited after vaccination in PI versus naive participants, with more proinflammatory and antiviral CD4+ and CD8+ T cell functional profiles in almost all PI individuals, including IFN-γ, TNF-α, and for the CD8+ T cells, cytotoxic functions. Based on current knowledge, we suggest that a balanced humoral and Th1-directed cellular immune response may be important for protection from COVID-19 and the development of effective vaccine-induced immunization.

S-specific CD4+ T cell responses clearly dominated over CD8+ T cell responses, both for AIM and ICS measurements. Because of their role in antigen-specific B cell survival and maturation, we studied the correlation of CD4+ T cell responses with Ab immunity. We found strong positive correlations between S-specific AIM+ CD4+ T cell responses measured before vaccination and isotype-switched IgA and IgG Ab responses after vaccination, as well as ADCC and neutralization functions, contrasting with no significant correlations with the unswitched IgM responses. These patterns suggest that pre-existing memory T cell help is a major modulator of humoral SARS-CoV-2 vaccine responses. While the patterns of predictive associations were overall similar for total AIM+CD4+ T cells and AIM+ cTfh, the correlations were weaker with ICS measurements. Again, this suggests that the widely used ICS assays likely miss CD4+ T cell subsets that are important to sustain the development of vaccine Ab responses. Consistent with our observations on robust cTfh induction by BNT162b2 mRNA, it was shown that SARS-CoV-2 mRNA vaccine had a superior capacity, in comparison to rBD-AddaVax, to elicit potent SARS-CoV-2-specific GC B cell responses after the administration of a single vaccine dose, suggesting that GC B cells and Tfh cells strongly correlated with the production of protective SARS-CoV-2-specific Ab responses (Lederer et al., 2020). Our results are also consistent with recent observations in convalescent COVID-19 donors, with reported correlations between antigen-specific CD4+ T cells (Grifoni et al., 2020) and cTfh cells (Mathew et al., 2020) and SARS-CoV-2-specific Abs. As the CD4 help-dependent development of high-affinity Ab responses is a desired outcome after vaccination, our results provide clear rationales for assessing CD4+ T cell responses as part of the evaluation of SARS-CoV-2 vaccine immunogenicity and durability of protection and for including function-agnostic techniques such as the AIM assays.

The availability of longitudinal sampling with six time points starting from a few weeks PSO up to 3 weeks post-vaccination enabled us to investigate the predictive capacity of distinct time points in infected/convalescent individuals for vaccine outcome in terms of humoral responses (Figure S5). At the earliest time point, a few weeks PSO, the predictive power of the studied parameters neutralization, ADCC, and ELISA binding
responses (IgA, IgG, IgM, and total Ig) were low; however, starting from time point 2, total Ig, IgG, and ADCC responses gain power to significantly predict stronger IgG responses post-vaccination. At the latest time point PSO, the predictive capacity of IgG and total Ig were partly diluted, but overall broadened, including predictions toward stronger IgA and IgM responses post-vaccination.

We note that vaccination of SARS-CoV-2 naive individuals with a mRNA vaccine bring their SARS-CoV-2-specific humoral and T cell responses to similar levels than the ones presented in individuals that were infected around nine months ago. Recent studies showed that natural infection confers up to 80% of protection from re-infection (Hall et al., 2021; Lumley et al., 2021); however, whether the same immune responses than those elicited by vaccination confer this protection remains unknown. Also, whether these humoral and T cell responses are unique to mRNA vaccine or could be extended to non-mRNA vaccines remains to be determined. These results give support to the consideration by various jurisdictions of a widened interval between the first and second dose in the context of vaccine shortage to protect a larger proportion of the population. The United Kingdom has decided to wait up to 12 weeks before administering the second dose of SARS-CoV-2 vaccines (Voysey et al., 2021), whereas Canada extended this interval up to 16 weeks (Public Health Agency of Canada, 2021). This is also advocated in the United States in the context of the surging B.1.1.7 variant (Osterholm et al., 2021).

While the duration of a protective immune response elicited by a single dose of mRNA vaccines is unknown, given that memory is a core function of the immune system, it is unlikely to decline within these intervals. Nevertheless, addressing this question will be very important as the larger the interval between doses is, the easier it will be to maximize the protection globally given the limited vaccine supply worldwide.

Limitations of study
Our results suggest that different antibody functions, beyond neutralization, could play a role in vaccine efficacy 3 weeks after a single dose of mRNA BNT162b2 vaccine in SARS-CoV-2 naive and PI individuals. A limitation of the study is that while we have shown that these humoral responses correlate with CD4 T cell responses, we don’t know in which extent CD4 and CD8 T cell responses directly contribute to vaccine efficacy, besides their helper role for antibody immunity. Additional studies will be required to understand the role of T cell-mediated immunity in mRNA vaccine efficacy.

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

- Human subjects
- Plasma and antibodies
- Cell lines

- METHOD DETAILS
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  - Cell-based ELISA
  - Cell surface staining and flow cytometry analysis
  - ADCC assay
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SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.chom.2021.06.001.

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AUTHOR CONTRIBUTIONS
A.T., M.N., D.E.K. and A.F. conceived the study. A.T., M.N., R.G., G.B.B., J.P., S.P.A., J.N., J.R., D.E.K., and A.F. designed experimental approaches. A.T., M.N., M.B., S.Y.G., R.G., G.B.B., N.B., O.T., J.P., S.P.A., C.B., J.R., D.E.K., and A.F. performed, analyzed, and interpreted the experiments. A.T., M.N., R.D., and O.T. performed statistical analysis. S.Y.G., A.L., J.P., H.M., G.G.L., G.G., L.S., A.T.M., M.U., M.L., W.M., J.R., and A.F. contributed unique reagents. L.G., C.M., P.A., V.M.-L., and C.T. collected clinical samples. C.L., G.D.S., S.M., and M.R. provided scientific input related to VOC. A.T., M.N., D.E.K., and A.F. wrote the manuscript with inputs from others. Every author has read, edited, and approved the final manuscript.
DECLARATION OF INTERESTS
The authors declare no competing interests.

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### STAR★METHODS

#### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|----------------------|--------|------------|
| **Antibodies**       |        |            |
| Antibodies           |        |            |
| UCHT1 (BUV395) [Human anti-CD3] | BD Biosciences | Cat#563546 ; Lot:9058566 ; RRID:AB_2744387 |
| UCHT1 (BUV496) [Human anti-CD3] | BD Biosciences | Cat#612941 ; Lot:1022424 ; RRID:AB_2870222 |
| L200 (BV711) [Human anti-CD4] | BD Biosciences | Cat#563913 ; Lot:03000025 ; RRID:AB_2738484 |
| SK3 (BB630) [Human anti-CD4] | BD Biosciences | Cat#564448 ; Lot:8288818 ; RRID:AB_2744362 |
| RPA-T8 (BV570) [Human anti-CD8] | Biolegend | Cat#746304 ; Lot:9133961 ; RRID:AB_2743629 |
| M5E2 (BV805) [Human anti-CD14] | BD Biosciences | Cat#563869 ; Lot:8256597 ; RRID:AB_2738456 |
| F50 (BV650) [Human anti-CD16] | Biolegend | Cat#755700 ; Lot:7086896 ; RRID:AB_396050 |
| HIB19 (APC-eFluor780) [Human anti-CD19] | Thermo Fisher Scientific | Cat#47-0199 ; Lot:2145095 ; RRID:AB_1582231 |
| HIB19 (BV480) [Human anti-CD19] | BD Biosciences | Cat#564448 ; Lot:8288818 ; RRID:AB_2744432 |
| H100 (PerCP Cy5.5) [Human anti-CD45RA] | BD Biosciences | Cat#563429 ; Lot:8332746 ; RRID:AB_2738199 |
| NCAM16.2 (BUV737) [Human anti-CD56] | Biolegend | Cat#563913 ; Lot:03000025 ; RRID:AB_2738199 |
| F50 (PerCP-eFluor710) [Human anti-CD69] | Thermo Fisher Scientific | Cat#46-0699-42 ; Lot:190361 ; RRID:AB_2573694 |
| F50 (BV650) [Human anti-CD69] | Biolegend | Cat#310934 ; Lot:B303462 ; RRID:AB_2563158 |
| H4A3 (BV786) [Human anti-CD107A] | BD Biosciences | Cat#563869 ; Lot:8144866 ; RRID:AB_2738458 |
| ACT35 (APC) [Human anti-CD134 (OX40)] | BD Biosciences | Cat#563473 ; Lot:1015537 ; RRID:AB_2738230 |
| 4B4-1 (PE-Dazzle 594) [Human anti-CD137 (4-1BB)] | Biolegend | Cat#500344 ; Lot:B2261476 ; RRID:AB_2564091 |
| TRAP1 (BV421) [Human anti-CD154 (CD40L)] | BD Biosciences | Cat#555700 ; Lot:7086896 ; RRID:AB_396050 |
| J25D4 (BV421) [Human anti-CD185 (CXCR5)] | Biolegend | Cat#502915 ; Lot:B285221 ; RRID:AB_493121 |
| B27 (PECy7) [Human anti-IFN-γ] | BD Biosciences | Cat#555700 ; Lot:7086896 ; RRID:AB_396050 |
| MO1-17H12 (PE-Dazzle594) [Human anti-IL-2] | Biolegend | Cat#500344 ; Lot:B2261476 ; RRID:AB_2564091 |
| JES3-9D7 (PE) [Human anti-IL-10] | BD Biosciences | Cat#554498 ; Lot:8198773 ; RRID:AB_395434 |
| eBio64CAP17 (eFluor660) [Human anti-IL-17A] | Thermo Fisher Scientific | Cat#50-7179-42 ; Lot:2151998 ; RRID:AB_11149126 |
| Mab11 (Alexa Fluor 488) [Human anti-TNF-α] | Biolegend | Cat#502915 ; Lot:B285221 ; RRID:AB_493121 |
| LIVE/DEAD Fixable dead cell | Thermo Fisher Scientific | Cat# L34960 |
| LIVE-DEAD Fixable AquaVivid Cell Stain | Thermo Fisher Scientific | Cat# P34957 |
| Mouse monoclonal anti-SARS-CoV-2 Spike (CR3022) | Dr M. Gordon Joyce | RRID:AB_2848080 |
| **CV3-25** | [https://www.biorxiv.org/content/10.1101/2021.03.23.436684v1](https://www.biorxiv.org/content/10.1101/2021.03.23.436684v1) | N/A |
| Peroxidase AffiniPure Goat Anti-Human IgA + IgG + IgM (H+L) | Jackson ImmunoResearch | Cat # 109-035-064 ; RRID:AB_2337583 |
| Peroxidase AffiniPure F(ab’)2 Fragment Goat Anti-Human Serum IgA, α chain specific | Jackson ImmunoResearch | Cat # 109-036-011 ; RRID: AB_2337592 |
**Continued**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Peroxidase AffiniPure Goat Anti-Human IgM, Fcγ, fragment specific | Jackson ImmunoResearch | Cat # 109-035-129; RRID:AB_2337588 |
| Alexa Fluor® 647 AffiniPure Goat Anti-Human IgA + IgG + IgM (H+L) | Jackson ImmunoResearch | Cat # 109-605-064; RRID:AB_2337886 |
| Cell Proliferation Dye eFluor™ 670 | Thermo Fisher Scientific | Cat # 65-0840-85 |
| Cell proliferation dye eFluor450 | Thermo Fisher Scientific | Cat # 65-0842-85 |

**Biological samples**

| SARS-CoV-2 naive donor blood samples | This paper | N/A |
| SARS-CoV-2 prior infection donor blood samples | This paper | N/A |

**Chemicals, peptides, and recombinant proteins**

| Dulbecco’s Modified Eagle’s medium (DMEM) | Wisent | Cat# 319-005-CL |
| Roswell Park Memorial Institute (RPMI) | Thermo Fisher Scientific | Cat# 61870036 |
| Penicillin/Streptomycin | Wisent | Cat# 450-201-EL |
| Fetal Bovine Serum (FBS) | VWR | Cat# 97068-085 |
| Bovine Serum Albumin (BSA) | Sigma | Cat# A7638 |
| Phosphate Buffered Saline (PBS) | ThermoFisher Scientific | Cat# 10010023 |
| Tween 20 | Sigma | Cat# P9416-100ML |
| Puromycin Dihydrochloride | Millipore Sigma | Cat# P8833 |
| Passive Lysis Buffer | Promega | Cat# E1941 |
| Freestyle 293F expression medium | Thermo Fisher Scientific | Cat# A14525 |
| D-Luciferin Potassium Salt | Thermo Fisher Scientific | Cat# L2916 |
| Formaldehyde 37% | Thermo Fisher Scientific | Cat# F79-500 |
| QuickChange II XL Site-Directed Mutagenesis Kit | Agilent | Cat# 200521 |
| ExpiFectamine 293 transfection reagent | ThermoFisher Scientific | Cat# A14525 |
| Western Lightning Plus-ECL, Enhanced Chemiluminescence Substrate | Perkin Elmer Life Sciences | Cat# NEL105001EA |
| Ni-NTA agarose | Invitrogen | Invitrogen |
| PepMix™ SARS-CoV-2 (Spike Glycoprotein) | JPT | Cat#PM-WCPV-S-1 |
| Staphylococcal Enterotoxin B (SEB) | Toxin technology | Cat#BT202 |

**Experimental models: Cell lines**

| HEK293T cells | ATCC | Cat# CRL-3216; RRID: CVCL_0063 |
| 293T-ACE2 cells | (Prévost et al., 2020) | N/A |
| FreeStyle 293F cells | ThermoFischer Scientific | Cat# R79007; RRID: CVCL_D603 |
| CEM.NKr CCR5+ cells | NIH AIDS reagent program | Cat# ARP-4376 |
| CEM.NKr CCR5+S cells | (Anand et al., 2021b) | N/A |
| HOS cells | ATCC | Cat# CRL-1543 |
| HOS.S cells | (Anand et al., 2021b) | N/A |

**Recombinant DNA**

| plNL4.3 R-E- Luc | NIH AIDS reagent program | Cat# 3418 |
| pcDNA3.1(+)-SARS-CoV-2 RBD | (Beaudoin-Bussières et al., 2020) | N/A |
| pCG1-SARS-CoV-2 Spike D614G | (Beaudoin-Bussières et al., 2020) | N/A |
| pCG1-SARS-CoV-1 Spike | (Hoffmann et al., 2013) | N/A |
| pCAGGS-OC43 Spike | (Prévost et al., 2020) | N/A |
| MERS-CoV Spike | (Park et al., 2016) | N/A |
| pCMV3-HCoV-HKU1 Spike | Sino Biological | Cat# VG40021-UT |
| pcDNA3.1 SARS-CoV-2 Spike B.1.1.7 | Genscript | N/A |
| pCG1-SARS-CoV-2 Spike D614G/E484K | This paper | N/A |
| pCG1-SARS-CoV-2 Spike D614G/N501Y | This paper | N/A |

(Continued on next page)
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
The published article includes all datasets generated and analyzed for this study. Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact Author (andres.finzi@umontreal.ca).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Ethics statement
This study was approved by the CRCHUM ethic committee in accordance with the Declaration of Helsinki (IRB protocol 19.381). Blood samples were obtained from donors who consented to participate in this research project at CHUM (IRB protocol 19.381). Plasma and PBMCs were isolated by centrifugation and Ficoll gradient, and samples stored at −80°C and in liquid nitrogen, respectively, until use.

Human subjects
No specific criteria such as number of patients (sample size), clinical or demographic were used for inclusion, beyond PCR confirmed SARS-CoV-2 infection in adults. Information related to their average age and gender is reported in Table 1.

Plasma and antibodies
Plasma from SARS-CoV-2 naive and PI donors were collected, heat-inactivated for 1 h at 56°C and stored at −80°C until ready to use in subsequent experiments. Plasma from uninfected donors collected before the pandemic were used as negative controls and used to calculate the seropositivity threshold in our ELISA, cell-based ELISA, ADCC and flow cytometry assays (see below). The RBD-specific monoclonal antibody CR3022 was used as a positive control in our ELISA, cell-based ELISA, and flow cytometry assays and was previously described (Anand et al., 2021a; Beaudoin-Bussières et al., 2020; Prévost et al., 2020). Horseradish peroxidase (HRP)-conjugated Abs able to detect all Ig isotypes (anti-human IgM+IgG+IgA; Jackson ImmunoResearch Laboratories) or specific for the Fc region of human IgG (Invitrogen), the Fc region of human IgM (Jackson ImmunoResearch Laboratories) or the Fc region of human IgA (Jackson ImmunoResearch Laboratories) were used as secondary Abs to detect Ab binding in ELISA and cell-based ELISA experiments. Alexa Fluor-647-conjugated goat anti-human Abs able 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% CO2 in Dulbecco’s modified Eagle’s medium (DMEM) (Wisent) containing 5% fetal bovine serum (FBS) (VWR) and 100 µg/mL of penicillin-streptomycin (Wisent). CEM.NKr CCR5+ cells (NIH AIDS reagent program) were maintained at 37°C under 5% CO2 in Roswell Park Memorial Institute
FreeStyle 293F cells (Invitrogen) were grown in FreeStyle 293F medium (Invitrogen) to a density of 1 × 10^6 cells/mL at 37°C with 8% CO₂ with regular agitation (150 rpm). Cells were transfected with a plasmid coding for SARS-CoV-2 S RBD using ExpiFectamine 293 transfection reagent, as directed by the manufacturer (Invitrogen). One week later, cells were pelleted and discarded. Supernatants were filtered using a 0.22 μm filter (Thermo Fisher Scientific). The recombinant RBD proteins were purified by nickel affinity columns, as directed by the manufacturer (Invitrogen). The RBD preparations were dialyzed against phosphate-buffered saline (PBS) and stored in aliquots at −80°C until further use. To assess purity, recombinant proteins were loaded on SDS-PAGE gels and stained with Coomassie Blue.

### METHOD DETAILS

#### Plasmids

The plasmids expressing the human coronavirus S of SARS-CoV-2, SARS-CoV-1 (Hoffmann et al., 2013, 2020), HCoV-OC43 (Prévost et al., 2020) and MERS-CoV (Park et al., 2016) or the SARS-CoV-2 S RBD (Beaudoin-Bussières et al., 2020) were previously reported. The HCoV-HKU1 S expressing plasmid was purchased from Sino Biological. SARS-CoV-2 S mutations were introduced using the QuikChange II XL site-directed mutagenesis protocol (Stratagene). The presence of the desired mutations was determined by automated DNA sequencing. The plasmid encoding the S of the B.1.1.7 variant was codon-optimized and synthesized by Genscript.

#### Protein expression and purification

FreeStyle 293F cells (Invitrogen) were grown in FreeStyle 293F medium (Invitrogen) to a density of 1 × 10^6 cells/mL at 37°C with 8% CO₂ with regular agitation (150 rpm). Cells were transfected with a plasmid coding for SARS-CoV-2 S RBD using ExpiFectamine 293 transfection reagent, as directed by the manufacturer (Invitrogen). One week later, cells were pelleted and discarded. Supernatants were filtered using a 0.22 μm filter (Thermo Fisher Scientific). The recombinant RBD proteins were purified by nickel affinity columns, as directed by the manufacturer (Invitrogen). The RBD preparations were dialyzed against phosphate-buffered saline (PBS) and stored in aliquots at −80°C until further use. To assess purity, recombinant proteins were loaded on SDS-PAGE gels and stained with Coomassie Blue.

#### Enzyme-linked immunosorbent assay (ELISA)

The SARS-CoV-2 RBD ELISA assay used was previously described (Beaudoin-Bussières et al., 2020; Prévost et al., 2020). Briefly, recombinant SARS-CoV-2 S RBD proteins (2.5 μg/mL), or bovine serum albumin (BSA) (2.5 μg/mL) as a negative control, were prepared in PBS and were adsorbed to plates (MaxiSorp Nunc) overnight at 4°C. Coated wells were subsequently blocked with blocking buffer (Tris-buffered saline [TBS] containing 0.1% Tween20 and 2% BSA) for 1 h at room temperature. Wells were then washed four times with washing buffer (Tris-buffered saline [TBS] containing 0.1% Tween20). CR3022 mAb (50 ng/mL) or a 1/250 dilution of plasma from SARS-CoV-2-naive or PI donors were prepared in a diluted solution of blocking buffer (0.1% BSA) and incubated with the RBD-coated wells for 90 min at room temperature. Plates were washed four times with washing buffer followed by incubation with secondary Abs (diluted in a diluted solution of blocking buffer (0.4% BSA)) for 1 h at room temperature, followed by four washes.

HRP enzyme activity was determined after the addition of a 1:1 mix of Western Lightning oxidizing and luminol reagents (Perkin Elmer Life Sciences). Light emission was measured with a LB942 TriStar luminometer (Berthold Technologies). Signal obtained with BSA was subtracted for each plasma and was then normalized to the signal obtained with CR3022 present in each plate. The seropositivity threshold was established using the following formula: mean of all SARS-CoV-2 negative plasma + (3 standard deviation of the mean of all SARS-CoV-2 negative plasma).

#### Cell-based ELISA

Detection of the trimeric SARS-CoV-2 S at the surface of HOS cells was performed by a previously-described cell-based enzyme-linked immunosorbent assay (ELISA) (Anand et al., 2021b). Briefly, parental HOS cells or HOS-S cells were seeded in 96-well plates (4 × 10⁴ cells per well) overnight. Cells were blocked with blocking buffer (10 mg/mL nonfat dry milk, 1.8 mM CaCl₂, 1 mM MgCl₂, 25 mM Tris [pH 7.5], and 140 mM NaCl) for 30 min. CR3022 mAb (1 μg/mL) or plasma from SARS-CoV-2 naive or PI donors (at a dilution of 1/250) were prepared in blocking buffer and incubated with the cells for 1 h at room temperature. Respective HRP-conjugated Abs were then incubated with the samples for 45 min at room temperature. For all conditions, cells were washed 6 times with blocking buffer and 6 times with washing buffer (1.8 mM CaCl₂, 1 mM MgCl₂, 25 mM Tris [pH 7.5], and 140 mM NaCl). HRP enzyme activity was determined after the addition of a 1:1 mix of Western Lightning oxidizing and luminol reagents (PerkinElmer Life Sciences). Light emission was measured with an LB942 TriStar luminometer (Berthold Technologies). Signal obtained with parental HOS was subtracted for each plasma and was then normalized to the signal obtained with CR3022 present in each plate. The seropositivity threshold was established using the following formula: mean of all SARS-CoV-2 negative plasma + (3 standard deviation of the mean of all SARS-CoV-2 negative plasma).

#### Cell surface staining and flow cytometry analysis

293T cells were transfected with full length S of different Betacoronavirus. 48 h post-transfection, S-expressing cells were stained with the CV3-25 Ab or plasma from SARS-CoV-2-naive or PI donors, prior and after vaccination (1/250 dilution). AlexaFluor-647-conjugated goat anti-human IgM+IgG+IgA Abs (1/800 dilution) were used as secondary Abs. The percentage of transduced cells (GFP+ cells) was determined by gating the living cell population based on viability dye staining (Aqua Vivid, Invitrogen). Samples were acquired on a LSRII cytometer (BD Biosciences) and data analysis was performed using FlowJo v10.7.1 (Tree Star). The seropositivity threshold was established using the following formula: (mean of all SARS-CoV-2 negative plasma + (3 standard deviation of the mean of all SARS-CoV-2 negative plasma).
**ADCC assay**

For evaluation of anti-SARS-CoV-2 antibody-dependent cellular cytotoxicity (ADCC), parental CEM.Nkr.CCR5+ cells were mixed at a 1:1 ratio with CEM.Nkr.SARS-CoV-2.S cells. These cells were stained for viability (AquaVivid; Thermo Fisher Scientific, Waltham, MA, USA) and cellular dyes (cell proliferation dye eFluor670; Thermo Fisher Scientific) to be used as target cells. Overnight rested PBMCs were stained with another cellular marker (cell proliferation dye eFluor450; Thermo Fisher Scientific) and used as effector cells. Stained target and effector cells were mixed at a ratio of 1:10 in 96-well V-bottom plates. Plasma from SARS-CoV-2 naïve or PI individuals (1/50 dilution) or monoclonal antibody CR3022 (1 μM) was added to the appropriate wells. The plates were subsequently centrifuged for 1 min at 300 g, and incubated at 37°C, 5% CO₂ for 5 h before being fixed in a 2% PBS-formaldehyde solution. ADCC activity was calculated using the formula: [(% of GFP+ cells in Targets + Effectors)−(% of GFP+ cells in Targets x 100] by gating on transduced live target cells. All samples were acquired on an LSRRII cytometer (BD Biosciences) and data analysis was performed using FlowJo v10.7.1 (Tree Star). The specificity threshold was established using the following formula: (mean of all SARS-CoV-2 negative plasma + 3 standard deviation of the mean of all SARS-CoV-2 negative plasma).

**Viral infectivity**

293T cells were transfected with the lentiviral vector pNL4.3 R-E- Luc (NIH AIDS Reagent Program) and plasmid encoding for the indicated S glycoprotein (D614G, B.1.1.7, D614G/E484K, D614G/N501S, D614G/S477N and D614G/N501Y) at a ratio of 5:4. Two days post-transfection, cell supernatants were harvested and stored at −80°C until use. The RT activity was measured by estimation of the incorporation of [methyl-³H]TTP into cDNA of a poly(A) template in the presence of virion-associated RT and oligo(dT). Normalized amount of RT activity pseudoviral particles were added to 293T-ACE2 target cells for 48 h at 37°C. Then, 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 MgSO₄, 15mM KPO₄ [pH 7.8], 1mM ATP, and 1mM dithiothreitol) and 50 μL of 1mM d-luciferin potassium salt (Thermo Fisher Scientific). RLU values obtained were normalized to D614G.

**Virus neutralization assay**

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, B.1.1.7, D614G/E484K, D614G/N501S, D614G/S477N and SARS-CoV-1) at a ratio of 5:4. Two days post-transfection, cell supernatants were harvested and stored at −80°C until use. 293T-ACE2 target cells were seeded at a density of 1 x 10⁶ cells/well in 96-well luminometer-compatible tissue culture plates (Perkin Elmer) 24 h before infection. Pseudoviral particles were incubated with the indicated plasma dilutions (1/50; 1/250; 1/1250; 1/6250; 1/31250) for 1 h at 37°C and were then added to the target cells followed by incubation for 48 h at 37°C. Then, 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 MgSO₄, 15mM KPO₄ [pH 7.8], 1mM ATP, and 1mM dithiothreitol) and 50 μL of 1mM d-luciferin potassium salt (Thermo Fisher Scientific). The neutralization half-maximal inhibitory dilution (ID₅₀) represents the plasma dilution to inhibit 50% of the infection of 293T-ACE2 cells by SARS-CoV-2 pseudoviruses.

**Intracellular cytokine staining**

PBMCs were thawed and rested for 2ch in RPMI 1640 medium supplemented with 10% FBS, Penicillin-Streptomycin (Thermo Fisher scientific, Waltham, MA) and HEPES (Thermo Fisher scientific, Waltham, MA). 2 x 10⁶ PBMCs were stimulated with a S glycoprotein peptide pool (0.5 μg/mL per peptide from JPT, Berlin, Germany) corresponding to the pool of 315 overlapping peptides (15-mers) spanning the complete amino acid sequence of the S glycoprotein.

Cell stimulation was carried out for 6 h in the presence of mouse anti-human CD107A, Brefeldin A and monensin (BD Biosciences, San Jose, CA) at 37°C and 5% CO₂. DMSO-treated cells served as a negative control. Cells were stained for aquavivid viability marker (Thermo Fisher scientific, Waltham, MA) for 20cm in at 4°C and surface markers (30cm, 4°C), followed by intracellular detection of cytokines using the IC Fixation/Permeabilization kit (Thermo Fisher scientific, Waltham, MA) according to the manufacturer’s protocol before acquisition on a Symphony flow cytometer (BD Biosciences, San Jose, CA). Abs used are listed in the Table S2. Stained PBMCs were acquired on Symphony cytometer (BD Biosciences) and analyzed using FlowJo v10.7.1 software.

**Activation-induced marker assay**

PBMCs were thawed and rested for 3h in 96-well flat-bottom plates in RPMI 1640 supplemented with HEPES, penicillin and streptomycin and 10% FBS. 1.7 x 10⁶ PBMCs were stimulated with a S glycoprotein peptide pool (0.5 μg/mL per peptide) for 15 h at 37°C and 5% CO₂. A DMSO-treated condition served as a negative control and SEB-treated condition (0.5cmin, 4°C) followed by intracellular detection of cytokines using the IC Fixation/Permeabilization kit (Thermo Fisher scientific, Waltham, MA) according to the manufacturer’s protocol before acquisition on a Symphony flow cytometer (BD Biosciences). Analyses were performed using FlowJo v10.7.1 software.
QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis
Symbols represent biologically independent samples from SARS-CoV-2 naive individuals (n = 24) and SARS-CoV-2 PI individuals (n = 24). 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. Differences in responses for the same patient before and after vaccination were performed using Wilcoxon matched pair tests. Differences in responses between naive and PI individuals were measured by Mann-Whitney tests. Differences in responses against the SARS-CoV-2 variants for the same patient were measured by Friedman 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. Line charts were created with Prism 8.4.3 using normalized data and Akima spline interpolation. For correlations, Spearman’s R correlation coefficient was applied. Statistical tests were two-sided and p < 0.05 was considered significant.

Software scripts and visualization
Normalized heatmaps were generated using the complexheatmap, tidyverse, and viridis packages in R and RStudio (R; RStudio). Normalizations were done per “Analysis group,” e.g., separately for all neutralization data, T cell responses, etc, except for binding analysis, which was normalized per individual parameter because different Abs are needed for the detection of Ig responses. IDs were grouped and clustered separately according to naive versus PI individuals, and also according to the time points before vaccination (V0) and after vaccination (V1). Squared correlograms were generated using the corrplot and RColorBrewer packages in program R and RStudio. Edge bundling graphs were generated in undirected mode in R and RStudio using ggraph, igraph, tidyverse, and RColorBrewer packages. Edges are only shown if p < 0.05, and nodes are sized according to the connecting edges’ r values. Nodes are color-coded according to groups of parameters. Area graphs were generated for the display of normalized time series. The plots were created in RawGraphs using DensityDesign interpolation and vertically un-centered values (Mauri et al., 2017). Line charts in overlay were created with Prism 8.4.3 using normalized data per response and Akima spline interpolation.