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Spike-specific T-cell responses in patients with COVID-19 successfully treated with neutralizing monoclonal antibodies against SARS-CoV-2

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ABSTRACT

Objectives: Neutralizing monoclonal antibodies (moAbs) improves clinical outcomes in patients with COVID-19 when administered during the initial days of infection. The action of moAbs may impair the generation or maintenance of effective immune memory, similar to that demonstrated in other viral diseases. We aimed to evaluate short-term memory T-cell responses in patients effectively treated with bamlanivimab/etesevimab, casirivimab/imdevimab, or sotrovimab (SOT).

Methods: Spike (S)-specific T-cell responses were analyzed in 23 patients with COVID-19 (vaccinated or unvaccinated) before and after a median of 50 (range: 28-93) days from moAb treatment, compared with 11 vaccinated healthy controls. T-cell responses were measured by interferon-γ-enzyme-linked immunospot and flow cytometric activation-induced marker assay.

Results: No statistically significant difference in S-specific T-cell responses was observed between patients treated with moAb and vaccinated healthy controls. Bamlanivimab/etesevimab and casirivimab/imdevimab groups showed significant increases in cellular responses in paired baseline/postrecovery series, as well as vaccinated patients receiving SOT. In contrast, unvaccinated patients prescribed SOT presented no statistically significant increases in T-cell-responses, suggesting diverse impacts of different moAbs on the evolution of S-specific T-cell responses in vaccinated and unvaccinated patients.

Conclusion: The moAbs did not hinder short-term memory S-specific T-cell responses in the overall group of patients; however, differences among moAbs must be further investigated both in vaccinated and unvaccinated individuals.

Introduction

Passive immunization by administering neutralizing monoclonal antibodies (moAbs) against SARS-CoV-2 is an effective therapeutic strategy in reducing both hospitalization and death related to COVID-19 (Dougan *et al.*, 2021; Gupta *et al.*, 2021; Montgomery *et al.*, 2022; Weinreich *et al.*, 2021). To date, the Food and Drug Administration has approved more than 30 SARS-CoV-2 moAbs for clinical trials. In Italy, five moAbs have been introduced into clinical practice for early treatment of COVID-19 following clearance by the Italian Drug Agency (AIFA) (AIFA, 2022). All these moAbs target the receptor-binding domain in the spike (S1) subunit of the viral S glycoprotein, each on distinct or partially overlapping epitopes. Most of the moAbs (e.g., bamlanivimab, etesevimab, casirivimab, imdevimab) recognize overlapping epitopes on the receptor-

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binding domain and receptor-binding motif (RBM), whereas sotrovimab (SOT) recognizes an epitope distal to the RBM and comprising a glycan moiety (Corti et al., 2021). The main mechanism of action of these moAbs is to hinder viral entry by blocking S1 engagement with the entry receptor angiotensin-converting enzyme (ACE2); however, direct inactivation of S protein has also been suggested (Lempp *et al.*, 2021). In addition to possessing neutralizing capacity, some SARS-CoV-2 moAbs can perform essential effector functions through their crystallizable fragments, such as antibody-dependent cell-mediated cytotoxicity and antibody-dependent cellular phagocytosis, thus promoting the killing of infected cells and providing adaptive immunity (Cathcart *et al.*, 2022).

Cellular immunity plays a critical role both in preventing SARS-CoV-2 infection and limiting disease progression (Merad et al., 2022; Moss, 2022; Sette and Crotty, 2021). Studies on convalescent patients with COVID-19 have shown that memory T-cell responses were robust and sustained up to 10 months after symptom onset (Adamo *et al.*, 2022; Jung *et al.*, 2021; Wheatley *et al.*, 2021), and that S-specific memory T-cell responses in BNT162b2 messenger RNA-vaccinated subjects appeared to be effective against viral variants (Geers *et al.*, 2021). However, major clinical determinants of poor outcomes, such as comorbidity and aging, may hinder the development of an effective protective cellular immunity against SARS-CoV-2 (Jing *et al.*, 2022). Recent studies have demonstrated that therapeutic antiviral moAbs can have an impact on host immune responses. In fact, moAbs against the respiratory syncytial virus can affect both humoral and cellular adaptive immune responses (Boyoglu-Barnum *et al.*, 2014), and anti-HIV-1 moAbs can boost viral antigen presentation through the so-called antibody vaccinal effect (Barouch *et al.*, 2013; Schoofs *et al.*, 2016). Engineered anti-influenza immunoglobulin (Ig) G moAbs can promote maturation of dendritic cells and protective cluster of differentiation 8+ (CD8+) T-cell responses (Bournazos et al., 2020), a finding that was exploited to optimize the SOT-derivative, VIR-7832 (Cathcart et al., 2022). Thus, based on previous experimental models, administration of moAbs in viral diseases could enhance (Barouch *et al.*, 2013; Boyoglu-Barnum *et al.*, 2014), hinder (Schmidt et al., 2020), or not interfere (Haigwood *et al.*, 2004; Jaworski *et al.*, 2013; Ng *et al.*, 2010) with the development of T-cell responses. As the degree of immune protection established after SARS-CoV-2 moAb treatment is of clinical interest in relation to the risk of reinfection (Sotoodeh Ghorbani et al., 2022) and time of vaccination (Centers for Disease Control and Prevention (U.S.), 2022), this study aimed to evaluate the onset of short-term memory T-cell response in a cohort of patients with COVID-19 treated effectively with bamlanivimab/etesevimab (BMT), casirivimab/imdevimab (REG), or SOT, highlighting any differences between these moAbs in promoting S-specific T-cell responses.

Methods

Study design

An observational longitudinal study was performed on 23 patients who were treated with moAbs from April 28, 2021 to March 28, 2022, according to the indications set by AIFA (AIFA, 2022), and who were available to provide us with a blood sample 1-3 months after symptom onset. Briefly, patients with COVID-19 symptoms for a maximum of 10 days who did not require oxygen support were prescribed moAbs provided that one or more of the following conditions were present: age >65 years, body mass index ≥30, chronic peritoneal dialysis or hemodialysis, uncontrolled or complicated diabetes mellitus, primitive or secondary immunodeficiency, cardiocerebrovascular disease, chronic obstructive pulmonary disease or other chronic respiratory disease, oncological or oncohematological diseases, chronic liver disease, hemoglobinopathies, or neurodegenerative disorder.

The first sample was collected on the same day of moAb administration (baseline, T1), that is, a median of 5 days after symptom onset (range: 1-11 days), and the second sample (after recovery, T2) was collected after a median of 50 days after symptom onset (range: 28-93 days). The second sample was collected from patients soon after the nasopharyngeal swab (NPS) became negative for SARS-CoV-2 RNA, and the NPS was collected weekly. Therapy with BMT, REG, or SOT was chosen for each patient based on drug availability, local epidemiology of SARS-CoV-2 variants of concern (De Marco *et al.*, 2022), and possible activity of moAbs against them (Lucas *et al.*, 2021). In fact, in the first period lasting from April 2021 to June 2021, the alpha variant appeared to be predominant (De Marco *et al.*, 2022), and BMT was prescribed in all six patients included in the current study, whereas in the second period lasting from July 2021 to March 2022, the delta and omicron variants were predominant and SOT was prescribed in most (14/18 = 78%) patients. Lastly, REG was prescribed in only three patients, two of whom required oxygen therapy, in accordance with the AIFA recommendation (AIFA, 2022; Weinreich *et al.*, 2021).

Before treatment at the COVID-19 University Center of Calabria Region, Italy (either as an outpatient or inpatient), the patients were evaluated by territorial medical services through molecular examination (*i.e.*, real-time polymerase chain reaction [PCR]) or third-generation rapid antigen nasal swab analysis for the detection of SARS-CoV-2 infection. Real-time PCR (GeneFinderTM COVID-19 Plus_RealAmp_Kit, Elitech Group) was performed on NPS collected on the same day of moAbs administration to confirm SARS-CoV-2 infection and on the day before the collection of the second blood sample to verify virological clearance.

Patients who did not receive any dose of vaccine and those who received only one dose of vaccine against SARS-CoV-2 were considered to be “not vaccinated” (Oberhardt *et al.*, 2021), whereas patients who received a single dose of the Ad26.COV2.S vaccine (Sadoff *et al.*, 2021), those who received two doses of the other types of vaccine 2-4 months before COVID-19 diagnosis, and those who received three doses of vaccine were considered to be “vaccinated”. Only one patient in this cohort received a single dose of Ad26.COV2.S vaccine.

A total of 11 healthy adult controls who did not exhibit any clinical evidence of current or previous SARS-CoV-2 infection presented with negative real-time PCR results for both NPS and serum anti-nucleocapsid Igs (Elecsys® Anti-SARS-CoV-2, Roche Diagnostics GmbH, Penzberg, Germany) and completed the three-dose schedule of Pfizer BNT162b2 vaccine in the previous 2-4 months were also enrolled.

Isolation of peripheral blood mononuclear cells

Peripheral venous blood was collected in ethylenediaminetetraacetic acid vacutainer tubes, and peripheral blood mononuclear cells (PBMCs) were isolated by density gradient isolation using Ficoll-Paque (Merck KGaA, Darmstadt, Germany). The isolated PBMCs were cryopreserved and stored in liquid nitrogen until use.

Enzyme-linked immunoSpot assay

Enzyme-linked immunospot (ELISpot) path kit (cod.3420-4AST-P1-1, Mabtech, Sweden) was used for the enumeration of PBMCs secreting interferon (IFN)-γ in response to peptides derived from the S protein of SARS-CoV-2 (S-pool). The detailed procedure is described in Supplementary data. The IFN-γ-ELISpot data were reported as immunospot forming units x10^6 PBMCs (stimulating forming unit (SFU)/10^6), which were calculated for each PBMC

sample by subtracting spots of the unstimulated wells from the spots of the cognate peptide-stimulated wells and normalizing to 10^6 PBMCs. The results were excluded if negative control wells had >30 SFU/10^6 PBMCs or positive control wells were negative.

Flow cytometry activation-induced cell marker assay and lymphocyte phenotype

The activation-induced cell marker (AIM) assay is a multiparametric flow cytometry method that allows the detection of CD4+ and CD8+ T-cells that are activated as a result of antigen-specific stimulation by upregulation of activation-induced surface markers (da Silva Antunes *et al.*, 2021; Grifoni *et al.*, 2021). The detailed procedure is described in Supplementary data. The AIM data were reported as stimulation index, which was calculated by dividing the percentage of AIM-positive cells after S-pool stimulation with the percentage of AIM-positive cells derived from the cognate DMSO stimulation point. For negative response in AIM, stimulation index was arbitrarily set to one to allow T1/T2 pairwise comparison.

Statistical analysis

Statistical analysis was performed by GraphPad Prism 9.0 Version 9.3.1 (GraphPad Software, San Diego, CA, USA), and the data are expressed as mean ± SEM, median, and percentage. Kruskal-Wallis test was used for multiple comparisons. Fisher's exact test was used to compare prevalence between the groups. The Mann-Whitney test was applied to compare unpaired continuous data not normally distributed, and the Wilcoxon matched-pairs signed rank test was used for T1/T2 pairwise comparison. We used the one-tailed hypothesis (T2 > T1) Wilcoxon matched-pairs signed rank test at the 0.05 level of significance (statistical power = 0.8). In this condition, the minimum sample size n = 5 was required to return a critical W-value at the level of significance we selected. For each test, $P < 0.05$ denoted statistical significance.

Ethical approval and consent to participate

The study was conducted according to the standards of the Declaration of Helsinki (revised in 2008) and was approved by the ethical committee of the Calabria Region (Protocol Reference: FESR/FSE 2014-2020 DDRC n. 585, Action 10.5.12, no-COVID19@UMG). Written informed consent was obtained from all the participants before moAbs administration and blood sample collection for the purpose of this study.

Results

Baseline (T1) characteristics of the enrolled patients

Among the 23 patients treated with moAbs, 12 were unvaccinated, and 11 had completed the vaccination schedule. These patients were compared with 11 healthy controls who had completed the vaccination schedule in the previous 2-4 months and did not report any clinical/serological proofs of current or previous SARS-CoV-2 infection. Among the patients with COVID-19, 21/23 (91.3%) patients with mild/moderate symptoms were treated early (within 10th day after symptom onset) with moAbs, whereas 2/23 (8.7%) patients required oxygen and received high-dosage REG according to international guidelines (Lucas *et al.*, 2021). There was no statistically significant difference in the percentage of CD19$^+$ B-cells, CD3$^+$ T-cells, CD4$^+$ CD3$^+$ T-cells, and CD8$^+$ CD3$^+$ T-cells between vaccinated and unvaccinated patients, and between each COVID-19 group and healthy controls. The characteristics of participants and immunological parameters at T1 are summarized in Table 1.

Comparison of spike-specific T-cell responses between vaccinated and unvaccinated patients at T1

We investigated the S-specific T-cell responses by measuring the frequency of IFN-γ-releasing cells (IFN-γ-ELISpot) and CD4$^+$ and CD8$^+$ T-cells expressing activation-induced markers (CD4$^+$AIM$^+$ and CD8$^+$AIM$^+$ cells, respectively) (da Silva Antunes *et al.*, 2021; Grifoni *et al.*, 2021) (Supplementary Figure S1). As these three T-cell-response markers are not redundant, we defined a positive T-cell response when at least one of these markers was positive. At T1, all the vaccinated patients with COVID-19 (11/11) and healthy controls (11/11) showed a positive S-specific T-cell response (Figure 1a). Among the vaccinated patients with COVID-19, only two cases exhibited undetectable anti-S IgG but positive S-specific T-cell response. In contrast, 10 of 12 (83.3%) unvaccinated patients with COVID-19 presented both undetectable S-specific IgG and negative T-cell response, whereas two cases showed both detectable S-specific IgG and a positive T-cell response (Figure 1a). At T1, the levels of all S-specific T-cell-response markers in the vaccinated COVID-19 group were not significantly different from those in the healthy controls but were, as expected, significantly higher than those in the unvaccinated COVID-19 group (Figure 1b and Supplementary Figure 1).

Comparison of spike-specific T-cell responses between vaccinated and unvaccinated patients at T2

We measured the postrecovery (T2) immunological parameters in unvaccinated (median: 47 days, range: 23-95 days after symptom onset) and vaccinated (median: 47 days, range: 28-93 days after symptom onset; $P = 0.7378$ Mann-Whitney test) patients. Collectively, the T-cell response rate at T2 for all patients with COVID-19 was 23/23 (100%) for IFN-γ-ELISpot, 15/23 (65.2%) for CD4$^+$AIM$^+$, and 16/23 (69.6%) for CD8$^+$AIM$^+$ (Figure 2a). The comparison between the unvaccinated and vaccinated patients with COVID-19 showed no statistically significant differences in the CD4$^+$AIM$^+$ response ($P > 0.9999$, Fisher's exact test) and CD8$^+$AIM$^+$ response ($P = 0.0686$, Fisher's exact test).

Pairwise comparison (T1/T2) of spike-specific T-cell responses between vaccinated and unvaccinated patients

The T1/T2 pairwise comparison of all patients with COVID-19 showed a significant increase in all S-specific T-cell-response markers (T2 vs T1 IFN-γ-releasing cells, $P < 0.0005$; T2 vs T1 CD4$^+$AIM$^+$, $P = 0.0471$) and CD8$^+$AIM$^+$ cells (T2 vs T1, $P = 0.0002$) (Figure 2b), reaching levels similar to those in healthy controls (Figure 2c). When stratified by vaccination status, the increased frequencies of all S-specific T-cell-response markers in the unvaccinated group at T2 were similar to those in healthy controls (Figure 3a,b). All the unvaccinated cases presented an increase in at least one T-cell-response marker at T2 (Figure 2A).

In the vaccinated group, both IFN-γ-releasing and CD8$^+$AIM$^+$ cells were significantly increased at T2; however, the levels of CD4$^+$AIM$^+$ cells were not significantly different between T2 and T1 (Figure 3c). The possible boosting effect of viral infection on pre-existing cellular immunity in vaccinated patients was particularly evident only in the CD8$^+$AIM$^+$ response, with the levels of CD8$^+$AIM$^+$ cells in vaccinated patients at T2 being significantly higher than those in both unvaccinated and healthy controls (Figure 3a). In particular, among the vaccinated patients, one patient (a man aged 73 years) presented no increase in any T-cell-response markers at T2. This patient was affected by low-grade noninfiltrating papillary urothelial bladder cancer, type 2 diabetes mellitus, hypertension, and hypercholesterolemia and had

Table 1
Characteristics of participants according to the vaccination status and immunological features.

		COVID-19 patients			Vaccinated healthy controls (N=11)
		Overall (N=23)	**Unvaccinated (N=12)**	**Vaccinated (N=11)**	
Age, median (range)		**66 (33-80)**	67 (33-78)	64 (45-80)	62 (35-72)
Female gender, N (%)		**5 (21.7%)**	3 (25%)	2 (18.2%)	4 (36.4%)
Comorbidities, N (%)					
	Diabetes mellitus	**5 (21.7%)**	2 (16.7%)	3 (27.3%)	-
	COPD and CCD	**5 (21.7%)**	3 (25%)	2 (18.2%)	-
	Oncohematological disease and/or immunodeficiency	**8 (34.8%)**	2 (16.7%)	6 (54.5%)	-
	Obesity	**3 (13%)**	3 (25%)	0 (0%)	-
	Other	**2 (8.7%)**	2 (16.7%)	0 (0%)	-
Type of monoclonal antibodies, N (%)					
	SOT	**13 (54.2%)**	5 (41.7%)	8 (71.7%)	-
	REG	**3 (13%)**	2 (16.7%)	1 (9%)	-
	BMT	**7 (30.4%)**	5 (41.7%)	2 (18.2%)	-
Immunological features					
Anti-spike IgG (BAU/ml), mean (SE)		**416 (170)**	5 (4)	423 (414)	568 (488)
Leukocytes, N/mm^3, mean (range)					
		5463 (2580-9740)	5874 (3010-9740)	5090 (2580-7650)	6060 (3540-7950)
Lymphocytes, N/mm^3, mean (range)					
		981 (250-1420)	1207 (880-1420)	777 (250-1370)	1180 (724-1420)
	CD19$^+$ B-cells (%PBMC), mean (range)	**3.1 (0.2-8.8)**	3.3 (0.6-8.8)	2.8 (0.2-8.2)	3.2 (0.4-7.2)
	CD3$^+$ T-cells (%PBMC), mean (range)	**53.2 (15.2-82.4)**	51.5 (15.2-82.4)	55.1 (24.4-77.7)	58.8 (34.1-87.6)
	CD4$^+$ T-cells (%PBMC), mean (range)	**46.5 (4.5-78.5)**	50.0 (12.6-78.5)	42.6 (4.5-74.7)	47.5 (11.5-78.3)
	CD8$^+$ T-cells (%PBMC), mean (range)	**23.2 (1.6-62.6)**	25.6 (10.2-51.5)	20.6 (1.6-62.6)	28.4 (8.6-56.6)

Abbreviations: BAU, binding antibody unit; BMT, bamlanivimab/etesevimab; CCD, cardiocerebrovascular diseases; CD, cluster of differentiation; COPD, chronic obstructive pulmonary disease; Ig, immunoglobulin; PBMC, peripheral blood mononuclear cells; REG, casirivimab/imdevimab; SOT, sotrovimab.

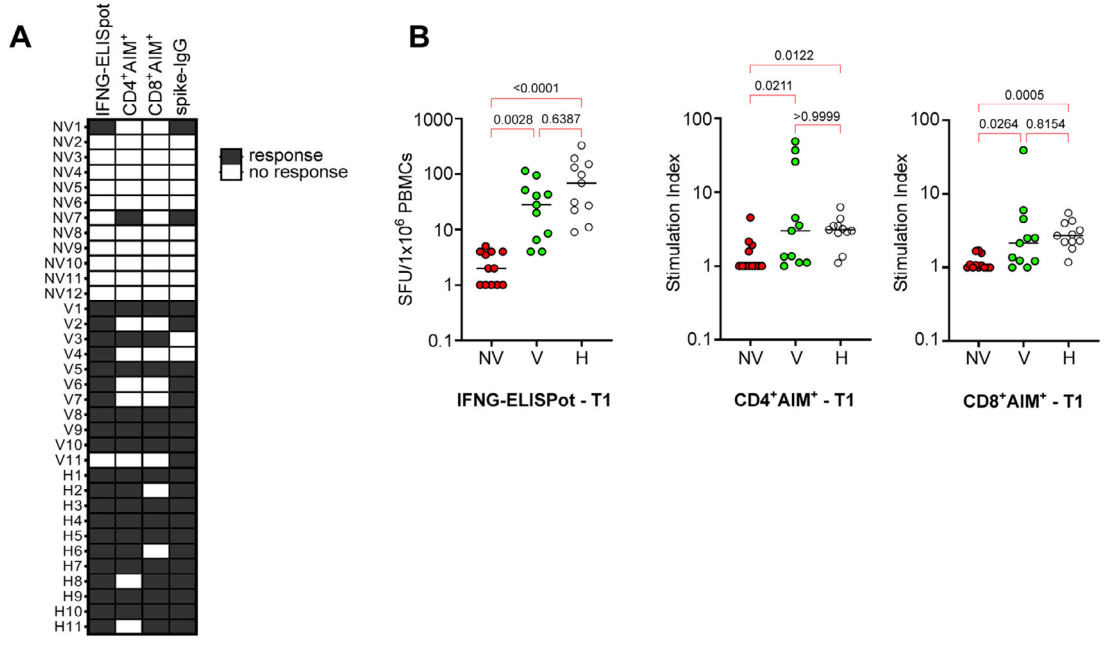

Figure 1. S-specific immune responses at T1. (a) Schematic summary of individual S-specific immune responses for COVID-19 NV, V, and H donors participants. (b) S-specific IFN-γ-releasing cells (left), AIM+(OX40+CD137+) CD4+ T-cells (middle), and AIM+(CD69+CD137+)CD8+ T-cells (right) measured after stimulation of PBMCs with peptides derived from the S protein of SARS-CoV-2. IFN-γ-releasing cells are reported as SFU/10^6 PBMCs. AIM+ responses are reported as stimulation index, calculated as described in methods. Statistical comparisons across groups were performed with the Kruskal-Wallis test. *P*-value is indicated for each pairwise comparison. Horizontal bars indicate the median.
Abbreviations: AIM, activation-induced cell marker; CD, cluster of differentiation; ELISpot, Enzyme-linked immunospot; H, healthy; IFN, interferon; Ig, immunoglobulin; NV, unvaccinated; PBMC, peripheral blood mononuclear cells; S, spike; SFU, stimulating forming unit; V, vaccinated.

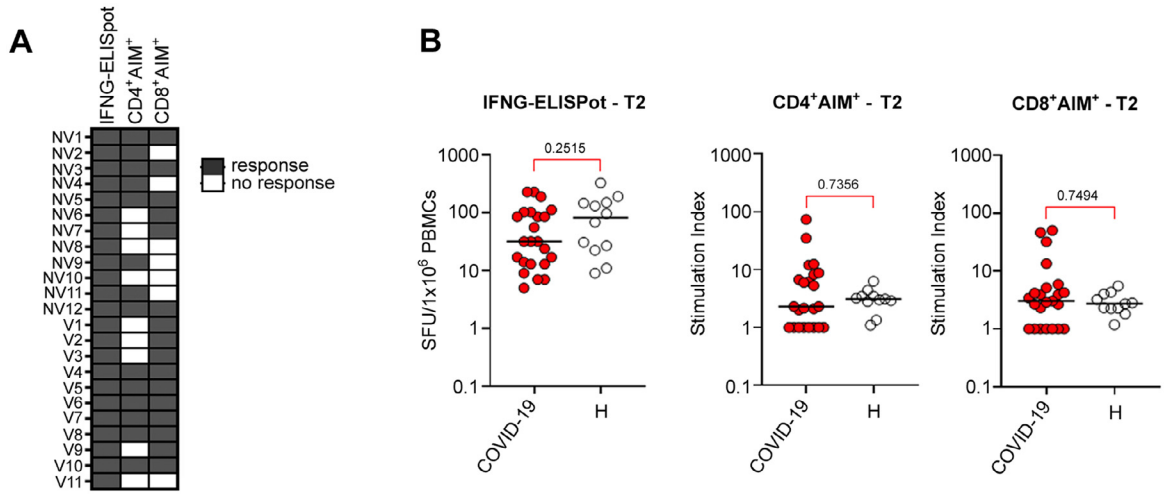

Figure 2. S-specific immune responses at T2. (a) Schematic summary of individual S-specific immune responses for NV and V COVID-19 participants. (b) S-specific IFN-γ-releasing cells (left), AIM+CD4+ T-cells (middle), and AIM+CD8+ T-cells at T2 in all COVID-19 patients and H donors. Statistical comparisons were performed with the Mann-Whitney test. *P*-value is indicated for each comparison. Horizontal bars indicate the median.
Abbreviations: AIM, activation-induced cell marker; CD, cluster of differentiation; ELISPOT, Enzyme-linked immunospot; H, healthy; IFN, interferon; NV, unvaccinated; PBMC, peripheral blood mononuclear cells; S, spike; SFU, stimulating forming unit; V, vaccinated.

been under mycophenolate and tacrolimus treatment after a kidney transplantation that occurred 17 years before.

Comparison of spike-specific T-cell responses between patients treated with different moAbs

The S-specific T-cell responses were compared between patients with COVID-19 treated with SOT (n = 13) and BMT/REG (n = 10). At T2, the IFN-γ-ELISpot response rate was 12/13 (92.3%) in the SOT group and 10/10 (100%) in the BMT/REG group (P >0.999, Fisher's exact test), the $CD4^+AIM^+$ response rate was 7/13 (53.8%) in the SOT group and 6/10 (60.0%) in the BMT/REG group (P >0.9999, Fisher's exact test), and the $CD8^+AIM^+$ response rate was 8/13 (61.5%) in the SOT group and 6/10 (60.0%) in the BMT/REG group (P >0.9999, Fisher's exact test). However, the levels of S-specific IFN-γ-releasing, $CD8^+AIM^+$, and $CD4^+AIM^+$ cells were not significantly different between the two treatment groups at T2 (Figure 4a).

In the T1/T2 pairwise comparison, both the treatment groups exhibited increased frequencies of IFN-γ-releasing cells (T2 vs T1 SOT, P = 0.0005; T2 vs T1 BMT/REG, P = 0.0020) and $CD8^+AIM^+$ cells (T2 vs T1 SOT, P = 0.0117; T2 vs T1 BMT/REG, P = 0.0156), whereas the frequencies of $CD4^+AIM^+$ cells were not significantly different (T2 vs T1 SOT, P = 0.1172; T2 vs T1 BMT/REG, P = 0.2188) (Figure 4b). Considering unvaccinated patients, the BMT/REG group (n = 7) showed a significant increase in the levels of all T-cell-response markers in the paired T1/T2 series (Figure 5a), whereas the SOT group (n = 5) did not present any statistically significant difference in all the S-specific T-cell-response markers (Figure 5b). In contrast, among vaccinated patients receiving SOT (n = 8), we observed a significant increase in both the IFN-γ-ELISpot and $CD8^+AIM^+$ responses but not in the $CD4^+AIM^+$ responses (Figure 6). The low number of patients treated with BMT/REG (n = 3) did not allow us to perform the statistical comparison of the paired T1/T2 series.

Discussion

This study evaluated short-term memory, and S-specific T-cell responses in patients with COVID-19 treated with anti-SARS-CoV-2 moAbs. Although a few previous works had investigated the humoral immune response in patients with COVID-19, to the best of our knowledge, the current study is the first to assess the effects of moAbs on cellular immune responses in patients with COVID-19 (Benschop *et al.*, 2022; Sasaki et al., 2022; Zhang *et al.*, 2021). In general, previous studies had reported only slight attenuations in both antiviral Ig levels and their neutralizing capacity. As assessment of humoral immunity alone is not sufficient for understanding the risk in terms of reinfection and long-term immune protection, in the current study, we measured the frequencies of IFN-γ-releasing, $CD4^+AIM^+$, and $CD8^+AIM^+$ cells, which are functional markers of T-cell responses evoked *in vitro* by stimulation with peptides derived from the S protein (da Silva Antunes *et al.*, 2021; Grifoni *et al.*, 2021). The higher frequency of IFN-γ-releasing cells, when compared with those of $CD4^+AIM^+$ and $CD8^+AIM^+$ cells, in T1/T2 pairwise comparison may be explained by the improved analytical sensitivity of the ELISpot method (Sette and Crotty, 2021), and by the fact that both $CD4^+$ and $CD8^+$ cells can release IFN-γ. Therefore, the frequency of S-specific IFN-γ-releasing cells may be a reliable biomarker of functional T-cell responses for assessment in larger prospective studies.

The study patients, predominantly with mild to moderate disease, were divided into two groups: vaccinated (had received two or three doses of vaccine) and unvaccinated. In accordance with the indications of the current guidelines on the use of SARS-CoV-2 therapeutic moAbs, the patients were heterogeneous with respect to comorbidities and were affected by immunosuppresive diseases or received immunosuppressive drugs. Despite this heterogeneity, the baseline levels of S-specific T-cell responses in the vaccinated group were similar to those observed in the triple-vaccinated healthy controls who had completed the vaccination schedule, including the booster dose, in an average of 85 days. Therefore, it is possible that the robust cellular responses observed in the present current were the consequence of the viral booster effect that occurred during the time between infection and sampling, superimposed on the S-specific T-cell responses in the vaccinated patients. These responses also occurred in two patients in the vaccinated group, who showed undetectable anti-S IgG. Although this is an anecdotal evidence in only two patients from a small group, it is consistent with previous studies that reported virus-specific cellular responses without evidence of virus-specific antibodies (da Silva Antunes *et al.*, 2021; Nelde *et al.*, 2021; Sekine *et al.*, 2020), suggesting immune protection induced by vaccines notwithstanding undetectability of IgG anti-S protein.

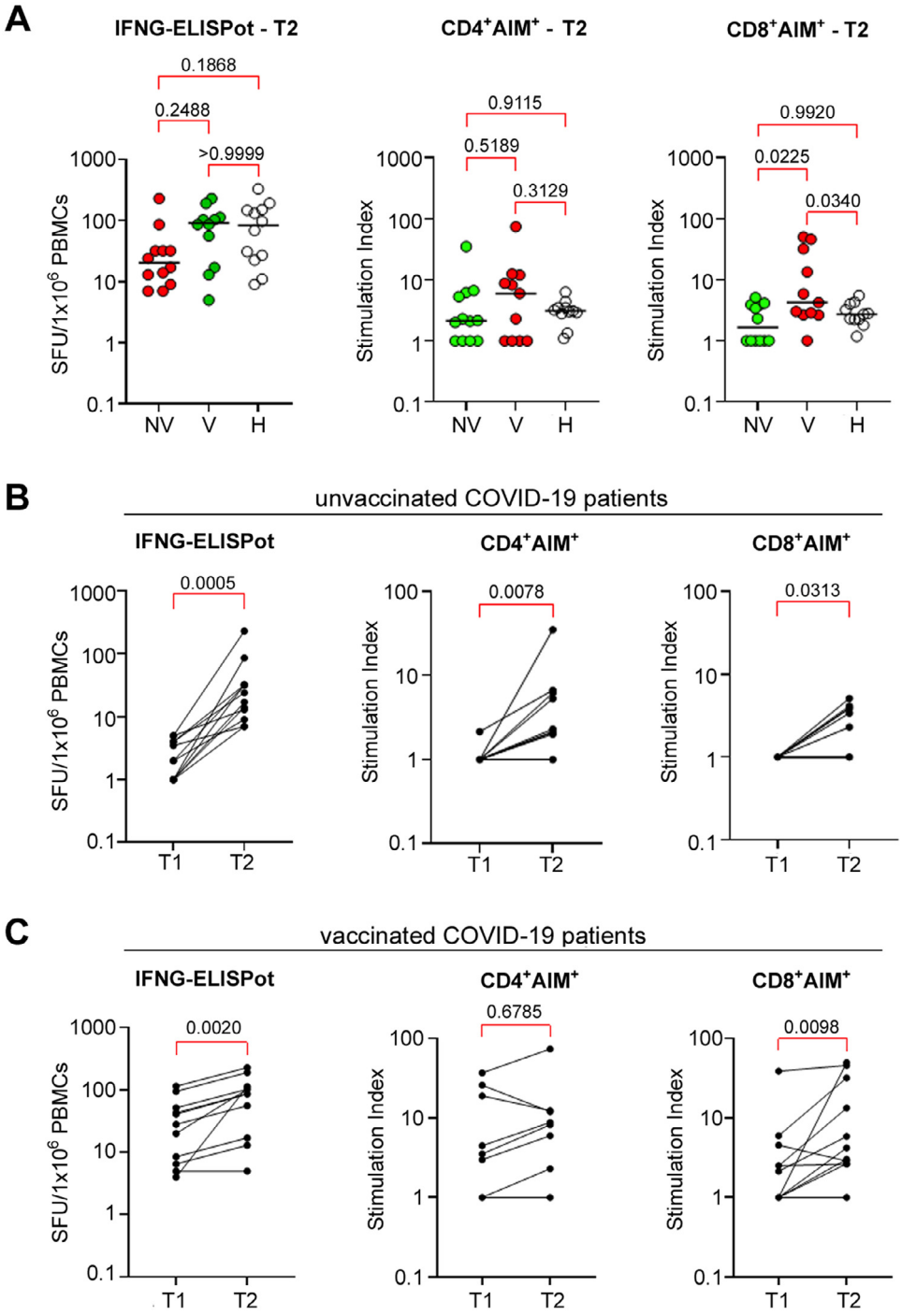

Figure 3. Comparison of S-specific T-cell responses between V and NV patients. (a) S-specific IFN-γ-releasing cells (left), AIM+CD4+ T-cells (middle), and AIM+CD8+ T-cells measured in NV, V, and H donors participants at T2. Statistical comparisons across groups were performed with the Kruskal-Wallis test. *P*-value is indicated for each pairwise comparison. Horizontal bars indicate the median. (b-c) T1/T2 pairwise comparison of S-specific IFN-γ-releasing cells (left), AIM+CD4+ T-cells (middle), and AIM+CD8+ T-cells measured in NV (b) and V (c) group. Statistical comparison for T1/T2 pairwise was performed by the Wilcoxon matched-pairs signed rank test. *P*-value is indicated for each comparison.

Abbreviations: AIM, activation-induced cell marker; CD, cluster of differentiation; ELISPOT, Enzyme-linked immunospot; H, healthy; IFN, interferon; NV, unvaccinated; PBMC, peripheral blood mononuclear cells; S, spike; SFU, stimulating forming unit; V, vaccinated.

The design of this study reasonably allowed us to distinguish two types of cellular responses: the *de novo* S-specific T-cell responses in unvaccinated patients and the boosted S-specific T-cell responses in vaccinated patients. From this perspective, it is possible to provide probable explanations for the differences in the cellular responses evoked in the short term after infection. In fact, in the current study, all the unvaccinated patients showed a significant increase in the frequencies of IFN-γ-releasing, $CD4^+AIM^+$, and $CD8^+AIM^+$ cells. Although these frequencies could not be compared with those determined in patients with COVID-19 not treated with moAbs, they were similar to those detected in triple-vaccinated healthy controls. Thus, even in subjects not previously vaccinated, moAbs treatment did not appear to hinder the establishment of a robust *de novo* S-specific T-cell responses.

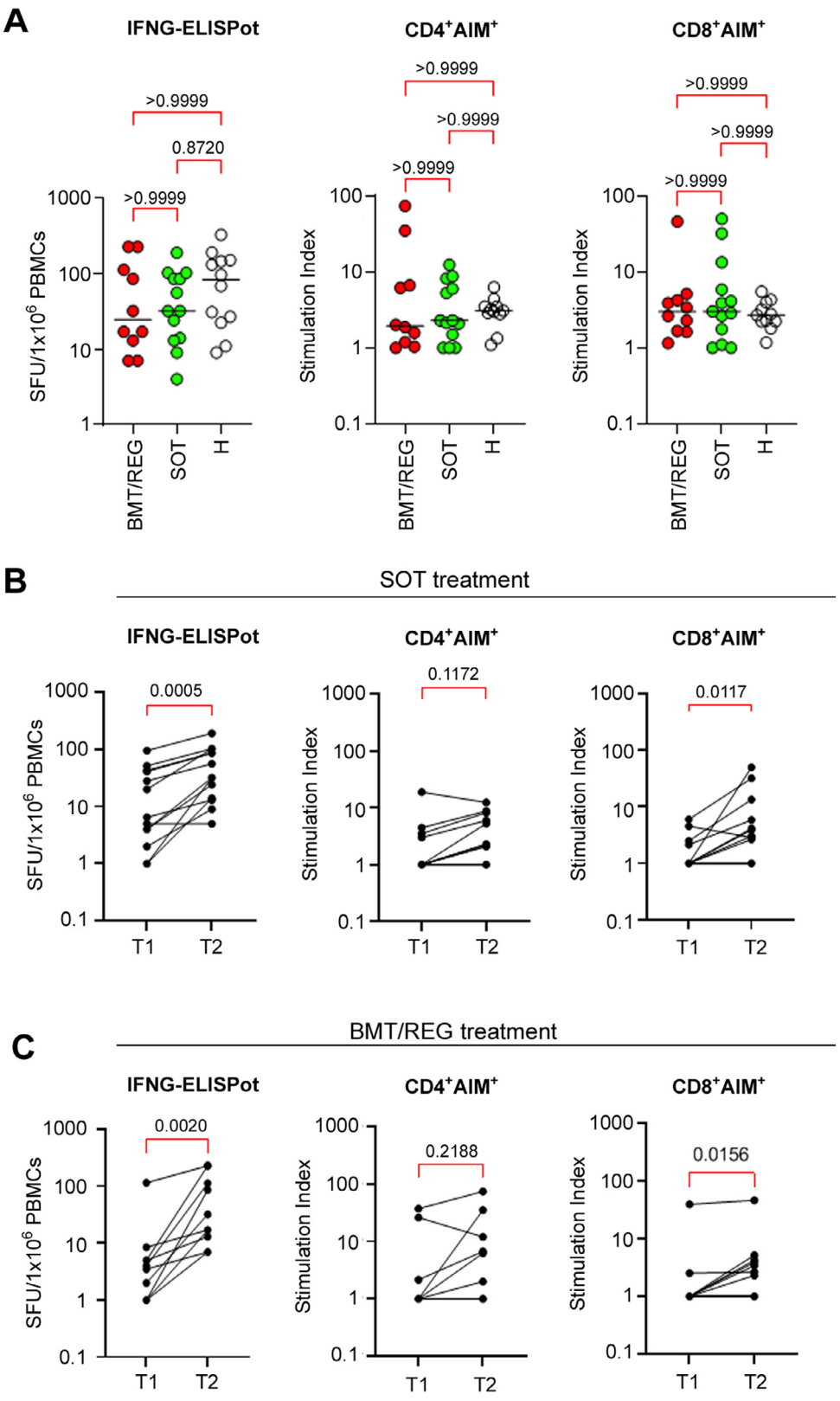

Figure 4. Comparison of S-specific T-cell responses between monoclonal antibodies treatment groups. (a) S-specific IFN-γ-releasing cells (left), AIM+CD4+ T-cells (middle), and AIM+CD8+ T-cells were measured in patients treated with BMT/REG, SOT, or in H-controls at T2. Statistical comparisons across groups were performed with the Kruskal-Wallis test. *P*-value is indicated for each pairwise comparison. Horizontal bars indicate the median. (b-c) T1/T2 pairwise comparison of S-specific IFN-γ-releasing cells (left), AIM+CD4+ T-cells (middle), and AIM+CD8+ T-cells measured in patients treated with SOT (b) or BMT/REG (c). Statistical comparison for T1/T2 pairwise was performed by the Wilcoxon matched-pairs signed rank test. *P*-value is indicated for each comparison.

Abbreviations: AIM, activation-induced cell marker;; CD, cluster of differentiation; BMT, bamlanivimab/etesevimab; ELISPOT, Enzyme-linked immunospot; H, healthy; IFN, interferon; PBMC, peripheral blood mononuclear cells; REG, casirivimab/imdevimab; S, spike; SFU, stimulating forming unit; SOT, sotrovimab.

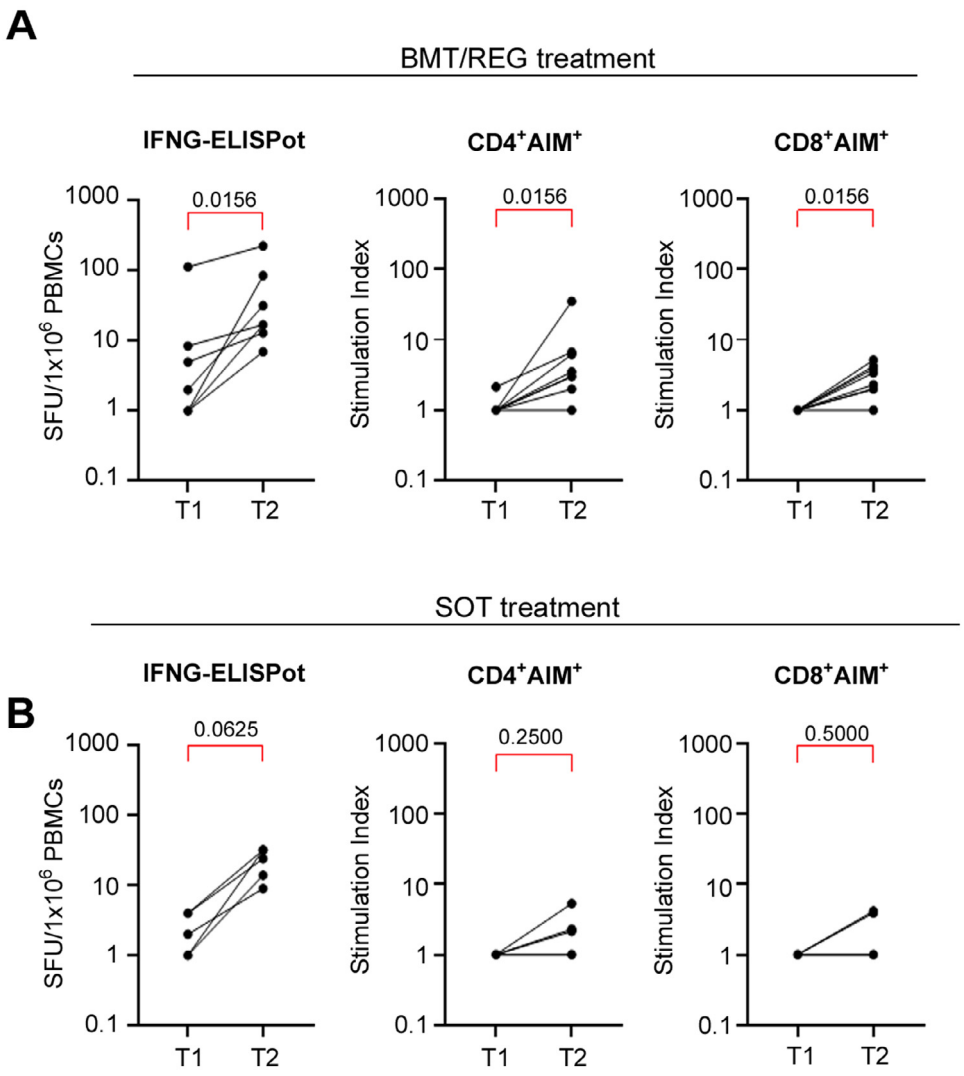

Figure 5. T1/T2 pairwise comparison of spike-specific IFN-γ-releasing cells (left), AIM+CD4+ T-cells (middle), and AIM+CD8+ T-cells measured in unvaccinated patients treated with BMT/REG (a) and SOT (b). Statistical comparison for T1/T2 pairwise was performed by the Wilcoxon matched-pairs signed rank test. *P*-value is indicated for each comparison.
Abbreviations: AIM, activation-induced cell marker; CD, cluster of differentiation; BMT, bamlanivimab/etesevimab; ELISPOT, Enzyme-linked immunospot; IFN, interferon; PBMC, peripheral blood mononuclear cells; REG, casirivimab/imdevimab; SFU, stimulating forming unit; SOT, sotrovimab.

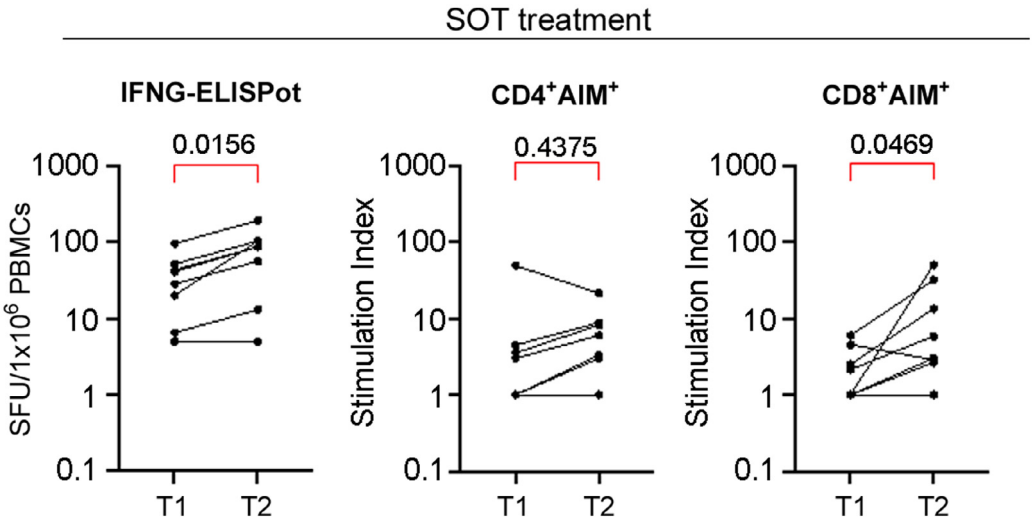

Figure 6. T1/T2 pairwise comparison of spike-specific IFN-γ-releasing cells (left), AIM+CD4+ T-cells (middle), and AIM+CD8+ T-cells measured in vaccinated patients treated with SOT. Statistical comparison for T1/T2 pairwise was performed by the Wilcoxon matched-pairs signed rank test. *P*-value is indicated for each comparison.
Abbreviations: AIM, activation-induced cell marker; ELISPOT, Enzyme-linked immunospot; IFN, interferon; SFU, stimulating forming unit; SOT, sotrovimab.

In the T1/T2 pairwise comparison, a significant increase in IFN-γ-releasing and $CD8^+AIM^+$ cells but not in $CD4^+AIM^+$ cells was observed in vaccinated patients; however, their levels were similar to those noted in the triple-vaccinated healthy controls.

In particular, the frequency of S-specific $CD8^+AIM^+$ cells in vaccinated patients was significantly higher than that in healthy controls, indicating a possible booster effect of SARS-CoV-2 infection on the pre-existing vaccine-induced immune response. However, only a few studies have reported on the S-specific T-cell responses after a breakthrough infection in vaccinated individuals (Blom *et al.*, 2022; Havervall *et al.*, 2022). For instance, Blom et al. (2022) demonstrated similar S-specific IFN-γ-releasing cells in triple-vaccinated participants without and with infection (7 weeks after breakthrough), consistent with the findings of the current study. Despite the role of moAbs treatment in increasing $CD8^+AIM^+$ frequency, the initial observation of boosted S-specific $CD8^+$ T-cell response in vaccinated patients reinforces the importance of vaccine protection notwithstanding a breakthrough infection. Regardless of the vaccination status and moAbs treatment, the levels of IFN-γ-releasing cells increased in all patients with COVID-19, except in one patient who received a kidney transplant and was taking mycophenolate and tacrolimus drugs. This patient did not show any significant increase in T-cell markers, which was correlated with the clinical progression of COVID-19. Although tacrolimus is not associated with severe disease or increased risk of death in patients with COVID-19 (Yin et al., 2021), mycophenolate can cause T-cell depletion and impairment of immune response to SARS-CoV-2 (De Santis *et al.*, 2022).

We conducted an exploratory analysis in the paired T1/T2 series stratified by status of vaccination. Although the analyzed group of patients is too small to form reliable conclusions, we observed that vaccinated patients in the BMT/REG group showed a significant increase in cellular responses, whereas no significant increase in cellular responses was noted in the SOT group. In contrast, among the vaccinated patients, the SOT group showed a significant increase in the IFN-γ-releasing and $CD8^+AIM^+$ but not in $CD4^+AIM^+$ cells. Although larger studies are needed to better characterize the evolution of T-cell responses after therapy with different moAbs, our results could be attributed to the different mechanisms of action of moAbs. For instance, SOT is a non-ACE2 moAbs blocker, which binds to an epitope distal to the RBM and consists of a glycan part. This epitope is involved in binding to the so-called attachment receptors, lectins DC-SIGN, L-SIGN, and SIGLEC1, which facilitate SARS-CoV-2 infection through the canonical ACE2 pathway (Corti et al., 2021). The role of these attachment receptors, prominently expressed on lung myeloid cells, explains the efficiency of lower respiratory tract infection, despite the paradoxically low level of ACE2 expression (Looney *et al.*, 2022). This finding indicated that besides the beneficial SARS-CoV-2-neutralizing effect of SOT and its capacity to normalize biomarkers that could predict severity and progression of SARS-CoV-2 infection compared with placebo (Maher et al., 2022), the blocking of lectin-facilitated infection could hinder the immune responses triggered by leukocyte cells, including lung myeloid cells. In support of this hypothesis, it has been recently suggested that lung myeloid cells may promote viral tissue dissemination and triggering of immune responses rather than being a direct target for productive infection (Lempp *et al.*, 2021).

A better understanding of the T-cell responses and their determinants after moAbs (including previous vaccination and type of moAbs) may have implications for optimal scheduling of SARS-CoV-2 vaccination after recovery from COVID-19 because the timing for vaccination after moAbs is still debated. Indeed, AIFA currently recommends to delay vaccination for COVID-19 for at least 3 months in patients who have received moAbs to avoid possible interference with the immune response induced by the vaccine (AIFA, 2022); in contrast, the US Centers for Disease Control and Prevention (Centers for Disease Control and Prevention (U.S.), 2022) does not indicate to delay COVID-19 vaccination after receipt of moAbs. However, if moAbs are confirmed to exhibit a different impact on the immune response, a targeted approach could be suggested. For instance, in patients receiving moAbs associated with a blunted immune response, vaccines should preferably be administered earlier than in those who were treated with moAbs with a neutral or a positive effect.

The first limitation of the current study was that our COVID-19 group was heterogeneous with respect to comorbidities, therapeutic treatments, and vaccination status, and the number of participants did not allow stratification or adjustment for data analysis. For instance, females were under-represented, and this is likely to have happened by chance due to the limited number of patients in the data collection. Second, the SARS-CoV-2 variants and subvariants were not characterized, and the number of participants was very small to determine reliable associations. However, at a population level, we were able to estimate the most prevalent variants in each period of this study (De Marco *et al.*, 2022), and the prescription of moAbs appeared to be consistent with providing the most effective therapy against SARS-CoV-2. Moreover, previous works have confirmed that cellular responses are maintained toward a broad spectrum of variants (Moss, 2022). Third, the current study did not specifically characterize the memory phenotypes of T-cells owing to the limited availability of biological samples. Lastly, because T2 samples were collected after performing a negative NPS in each patient, the correlation between T-cell responses and the outcomes of moAbs therapy could not be determined because of the lack of control.

Conclusion

Using a heterogeneous cohort of patients with COVID-19, the current study found that moAbs administration did not hinder the development of S-specific T-cell response in the short term, both in vaccinated and unvaccinated patients. In future research, the possible differences among moAbs should be further investigated.

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Author contributions

SR, EV, CT, and CP contributed to study conception, drafting, and critical revisions of the manuscript; AA, CG, SM, MTT, CC, AR, EMT, GC, and FSC made critical revisions and provided important intellectual content. All the authors have approved the final version of the manuscript.

Conflict of interest

The authors have no competing interests to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.09.016.

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