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SHORT COMMUNICATION

A single dose of COVID-19 vaccine induces a strong T cell and B cell response in healthcare professionals recovered from SARS-CoV-2 infection

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Abstract

A broad understanding on how SARS-CoV-2 infection and vaccination mobilize the immune system is necessary to find the best predictors of long-term protection and identify individuals that would benefit from additional vaccine doses. This study aims to understand the effect of a single dose of Pfizer-BioNTech BNT162b2 COVID-19 vaccine, in individuals recovered from SARS-CoV-2 infection, on circulating $CD4^+$ T follicular helper (Tfh)-cells, Spike-specific T-cells and IgG/IgA antibodies. For that, peripheral blood samples from 50 healthcare professionals, recovered from SARS-CoV-2 infection, collected immediately before (T1) and 15 days after (T2) vaccine administration, were used to analyze the frequency and numbers of Tfh-cells and their subsets, serum titers of SARS-CoV-2-specific antibodies, and SARS-CoV-2-specific T-cells. Six months after infection (T1), 96% of recovered participants presented either IgG or T-cells specific for Spike, however, Spike-specific T-cells were missing in 16% of them. These individuals presented lower levels of Spike-specific IgG (T1 and T2), IgA (T1), and Spike-specific T-cells (T2). Vaccination increased the percentage of participants reactive for Spike-specific T-cells (from 64 to 98%), IgG (from 90 to 100%) and IgA (from 48 to 98%). It also mobilized circulating Tfh-cells, increasing their frequency and activation, and promoting Tfh17 polarization, restoring the decreased numbers of Tfh-cells (especially Tfh17) observed in recovered participants. Interestingly, Tfh percentage correlated with Spike-specific IgG levels. Our data showed that a single dose of vaccine efficiently restored Spike-specific T-cells, and IgG and IgA antibodies. Mobilization of Tfh-cells, and their correlation with IgG levels, suggest that vaccination induced a functional Tfh cell response.

Keywords SARS-CoV-2 · COVID-19 · Vaccination · T follicular cells · Spike-specific T cells · Spike-specific IgG

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Introduction

The clinical course of coronavirus disease 2019 (COVID-19) displays a broad variation, from asymptomatic to severe pneumonia and death. Some comorbidities associated to severe disease are also characterized by an altered immune function [1–3]. The dysregulation of the immune response may affect the ability to generate T cells and antibodies with specificity for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigens, which has implications on long-term immune protection. The few studies carried out on the SARS-CoV-2 phylogenetically related virus, SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), point to a long-term immunity mainly dependent on memory T cells, rather than antibodies or memory B cells [4–6]. Moreover, for SARS-CoV-2, some data show that Spike-specific T cells maintain a robust response to different variants of the virus, while antibodies display a reduction on their neutralizing capability [7]. Based on this information, the development of an appropriated T cell specific immune response assumes a critical importance for COVID-19 pandemic.

$CD4^+$ T follicular helper (Tfh) cells assume particular relevance in this context because they link the cellular and humoral immune response, as they are crucial for memory B cells and plasma cells development, and regulate somatic hypermutation and immunoglobulin (Ig) class switching [8]. Circulating SARS-CoV-2 Spike-specific Tfh cells are detected in infected patients [9]. Nevertheless, this T cell subset displays a decreased percentage of cells, and an altered phenotype and function in SARS-CoV-2 infected, convalescent, or recovered individuals [10–13]. COVID-19 patients also present an extreme reduction in the formation of germinal centers in thoracic lymph nodes, when compared to other types of pneumonia [14, 15]. Accordingly, the altered phenotype/function of Tfh cells detected in COVID-19 patients may hamper their ability to effectively support antibody production, which can be on the basis of the weak humoral response against the virus observed in some patients [10]. These features are compatible with the limited somatic hypermutation and short-lived humoral immunity observed in COVID-19 [15].

This study seeks for a correlation between the frequency of circulating Tfh cells/ activated Tfh cells and SARS-CoV-2-specific IgG levels in individuals recovered from SARS-CoV-2 infection. Further, it aims to understand how a single dose of Pfizer-BioNTech mRNA COVID-19 vaccine impact circulating Tfh cells, and the amount of Spike-specific T cells, and Spike-specific IgG and IgA antibodies.

Material and methods

Participants and samples

This study enrolled 50 healthcare professionals (41 female, 9 male; mean age: 49.0 ± 6.1 years old, ranging from 40 to 61 years old), from Centro Hospitalar e Universitário de Coimbra (CHUC), Portugal, recovered from previous SARS-CoV-2 infection, and included in the Portuguese COVID-19 vaccination program. These participants received a single dose of Pfizer-BioNTech BNT162b2 mRNA COVID-19 vaccine, as recommended by the Portugal's Directorate-General of Health (Direção-Geral da Saúde, DGS). From these participants, 25 had asymptomatic disease or mild symptoms, whereas 25 had severe respiratory symptoms; time since diagnosis ranged from 185 to 433 days (mean: 336 ± 98 days), and time since recovering from 163 to 408 days (mean: 301 ± 88 days). A control group of 15 infection-naive (i.e., individuals that have never been infected with SARS-CoV-2) gender and age-matched donors (12 female, 3 male; mean age: 49.5 ± 5.6 years old, ranging from 41 to 61 years old) was also included, in order to evaluate the basal levels of the parameters under study.

A total of 20 ml of peripheral venous blood (PB) was collected from each participant, at Occupational Medicine Service from CHUC. For the group of healthcare professionals recovered from SARS-CoV-2 infection, blood was collected at 2 time points: immediately before the administration of the vaccine (T1) and 15 days after the administration of the vaccine (T2). Peripheral blood was used for Tfh cell analysis by flow cytometry, to measure the serum titers of SARS-CoV-2 specific antibodies by chemiluminescent microparticle immunoassay, and to determine the presence of SARS-CoV-2-specific T cells by T-Spot assay.

The study was approved by CHUC's Ethics Committee (OBS.SF.107–2021), and all participants gave their signed informed consent before entering the study.

Analysis of Spike- and NP-specific T cells by T-SPOT

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation with Lymphoprep (Stemcell Technologies, Vancouver, Canada), from heparin-collected PB, and used for the detection of Spike- and Nucleocapsid Protein (NP)-specific T cells, using T-SPOT. COVID test (Oxford Immunotec, Oxfordshire, UK), following the manufacturer's recommendations. A number of 250,000 PBMCs were consistently placed in each well of the plate, as recommended. The results were interpreted as indicated by the manufacturer: a final number of spot

forming cells (SFC) ≥ 8 (per 250,000 PBMCs) was considered reactive for Spike or NP-specific T cells, SFC ≤ 4 was considered non-reactive, and a number of SFC between 5 and 7 was considered borderline/equivocal.

Quantification of Spike- and NP-specific IgG in serum by chemiluminescent microparticle immunoassay

Serum from participants was processed with the chemiluminescent microparticle immunoassay (CMIA) SARS-CoV-2 IgG II Quant to determine IgG anti-Spike, receptor-binding domain (RBD), S1 subunit of SARS-CoV-2, on Alinity i (Abbott Laboratories). As per manufacturer recommendations, antibody titers above 50 AU/ml were considered reactive.

CMIA SARS-CoV-2 IgG anti-NP was used for the detection of SARS-CoV-2 IgG anti-NP antibodies, on Alinity i (Abbott Laboratories). As per manufacturer recommendations, antibody titers above or equal to 1.4 index (sample calibrator index ratio, S/C) were considered reactive.

All measurements were undertaken following appropriate quality control procedures, daily performed for routine clinical assessment of SARS-CoV-2 IgG antibodies.

Quantification of Spike-specific IgA in serum by ELISA

The semiquantitative determination of Spike-specific IgA serum levels was performed using the Anti-SARS-CoV-2 ELISA (IgA) kit from Euroimmun Medizinische Labordiagnostika AG (Lubek, Germany), following the manufacturer's instructions. The semiquantitative determination of Spike-specific IgA was made by the calculation of a ratio from the extinction of the sample and that of the calibrator, wherein a ratio ≥ 1.1 is positive, a ratio < 0.8 is negative, and borderline for ratios ≥ 0.8 to < 1.1.

Analysis of CD4$^+$ T follicular helper cells by flow cytometry

The identification and quantification of CD4$^+$ T follicular helper (Tfh) cells was performed by flow cytometry, using a stain-lyse-wash protocol, previously described [16]. EDTA-collected PB cells were stained with the following monoclonal antibodies (mAbs): CD185 (CXCR5) BV421, CD4 BV510, TCRγδ FITC, CD25 PE, HLA-DR PerCPCy5.5, CD8 PE-Cy7, CD127 AF-647, CD3 APC-H7, CD45RA FITC, CD196 (CCR6) PE, CD27 PerCPCy5.5, and CD195 (CCR5) APC. All mAbs were purchased from BD Pharmingen (San Diego, USA), except CD185, CD4, and TCRγδ, purchased from Becton Dickinson Biosciences (BD, San Jose, USA). The samples were immediately acquired in a FACSCanto II (BD) flow cytometer, and the number of events acquired was always above 0.5×10^6. Tfh cells were identified as CD3$^+$CD4$^+$CD8$^-$TCRγδ$^-$CD185$^+$ (Fig. 1) and, within Tfh cells, the following subsets were further identified: activated Tfh cells (CD25$^+$ and HLA-DR$^+$); naive (CD45RA$^+$CD27$^+$) and central memory (CD45RA$^-$CD27$^+$) Tfh cells with Tfh1 (CD195$^+$CD196$^-$), Tfh17 (CD195$^-$CD196$^+$), or Tfh1/Tfh17 (CD195$^+$CD196$^+$) phenotype, and CD195$^-$CD196$^-$ Tfh cells (Fig. 1). The identification of CD25 and HLA-DR positive cells was made based on internal negative control.

Statistical analyses

Data are presented as the mean values ± standard deviation. Paired-sample t-test and McNemar test were used to compare T1 vs. T2; t-test and chi-square were used to assess differences between symptomatic and asymptomatic individuals; population distribution normality was evaluated by the Kolmogorov–Smirnov test; the equality of variances was tested by Levene's test; Mann–Whitney test was used to compare reactive vs. non-reactive individuals for T-Spot, and infection-naive donors vs. participants recovered from infection at T1; Pearson's correlation was performed to seek for correlations among the parameters under study. Statistic analyses were performed using the Statistical Package for Social Sciences software (SPSS, version 27, IBM, Armonk, NY, USA). Statistical significance was considered when $p < 0.05$.

Results and Discussion

Almost all recovered individuals present specific immunity against SARS-CoV-2 six months after infection, but Spike-specific T cells are missing in 16% of them

For all recovered individuals enrolled in this study, the elapsed time since SARS-CoV-2 infection was, at least, 6 months. Six months after infection, 96% of the recovered participants displayed either IgG or T cells specific for Spike. This result contrasts with the smaller percentage of recovered individuals presenting either T cells or IgG specific for NP (66%). Interestingly, the percentage of recovered individuals presenting T cells with specificity for Spike and NP was similar at T1 (62%), in opposition to IgG antibodies, where 90% of recovered individuals displayed Spike-specific IgG, but only 30% had NP-specific IgG, at T1 (Table 1). This difference is expected because Spike is located on the virus surface, therefore is more prone to be targeted by antibodies, whereas NP is located within the viral envelope [17].

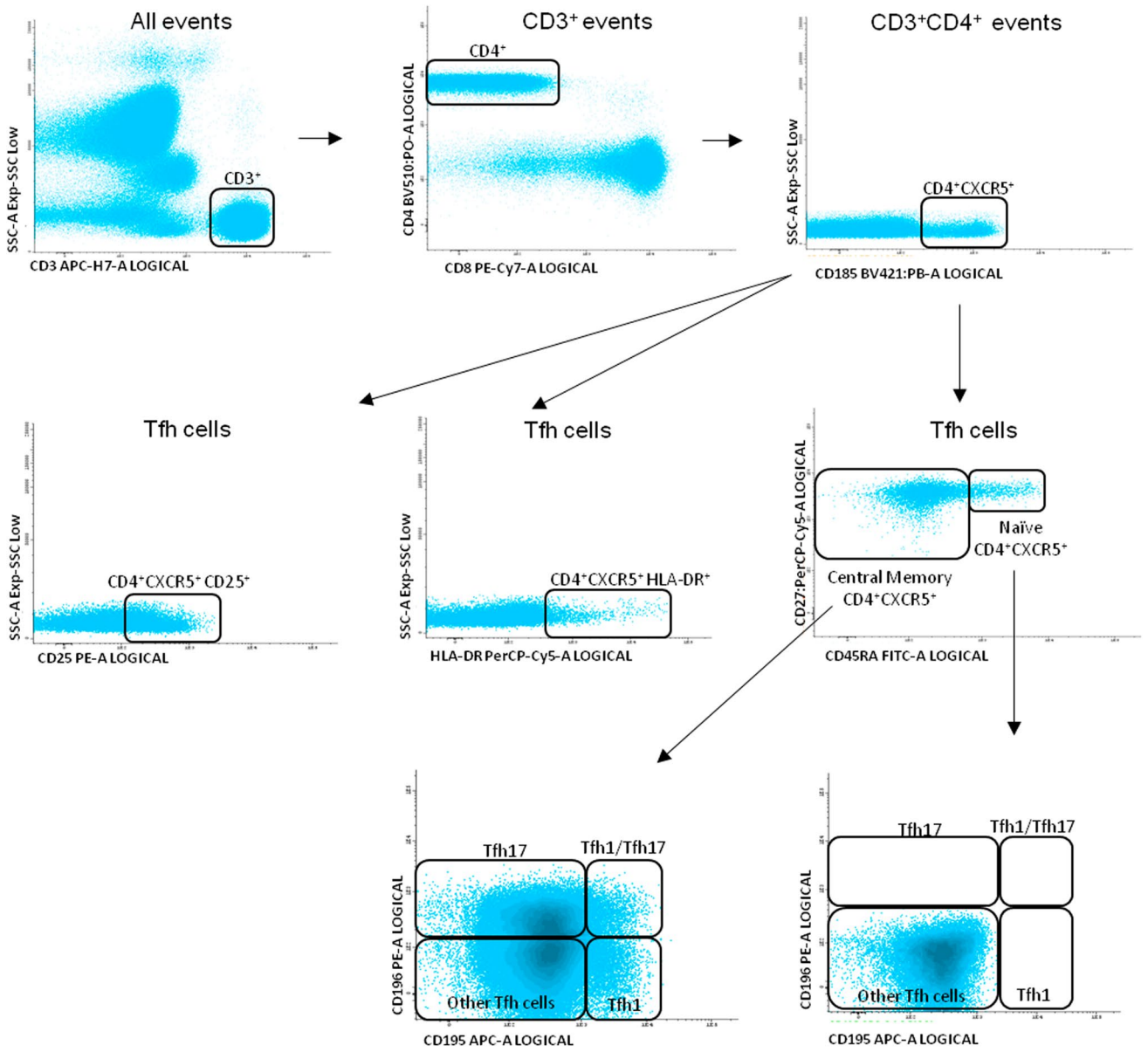

Fig. 1 Representative dotplots showing the strategy used for the identification of $CD4^+$ T follicular helper (Tfh) cell subsets; $CD3^+$ events correspond to *T* cells; Tfh cells were identified as $CD3^+CD4^+CD8^-CXCR5^+$; SSC, side scatter light dispersion

Considering the eight recovered individuals non-reactive for Spike-specific T cells (16% of all recovered participants), 7 (88%) of them were also non-reactive for NP-specific T cells. When comparing the non-reactive vs. reactive recovered participants for Spike-specific T cells at T1, significant differences were observed in the time since diagnosis (406 ± 46 and 327 ± 98 days, respectively), time since recovering (365 ± 43 and 292 ± 87 days, respectively), IgG Spike-specific antibody titers (85 ± 110 and 548 ± 662 AU/ml, at T1; and 16,300 ± 13,361 and 42,929 ± 23,156 AU/ml, at T2, respectively), and the level of Spike-specific T cells achieved after the vaccine administration, at T2 (39 ± 23 and 102 ± 82 SFC, respectively). In contrast, no differences were found for Spike-specific IgA levels or Tfh cell populations.

These results point to a loss of cellular immunity after natural infection. The contradictory results has been published concerning the longevity of SARS-CoV-2-specific T cells developed in the context of an infection, ranging from 3 to 5 months to 10 months in different studies [18, 19]. In this context, it is of utmost importance to evaluate and compare the longevity of SARS-CoV-2-specific T cells formed in the context of vaccination, both in recovered and in infection-naive individuals. Our results showed that 6 months after infection some individuals are missing Spike-specific T

Table 1 Frequency of recovered individuals presenting reactive T cells (assessed by T-Spot), serum IgG for Spike and NP, and serum IgA for Spike; and quantification of the levels of Spike- and NP-specific T cells, IgG and IgA, before (T1) and after (T2) COVID-19 vaccination

	COVID-19 symptoms	T1	T2
% individuals with Spike-specific T cells		62%* (31 out of 50)[a]	98% (48 out of 49)[b]
% individuals with Spike-specific IgG		90%	100%
% individuals with Spike-specific IgA		48%*	98%
% individuals with Spike-specific T cells or IgG[c]		96%	100%
% individuals with NP-specific T cells		62% (31 out of 50)[d]	61% (30 out of 49)[e]
% individuals with NP-specific IgG		30%	30%
% individuals with NP-specific T cells or IgG[c]		68%	70%
Spike-specific T cells (SFC) (mean ± standard deviation)[f]	Total	17 ± 14.6*	90 ± 70.2
	Asymptomatic	16 ± 9.7*	87 ± 65.0
	Symptomatic	17 ± 18.2*	93 ± 76.8
Spike-specific IgG (A.U./ml) (mean ± standard deviation)[g]	Total	447 ± 575*	36,534 ± 22,641
	Asymptomatic	462 ± 671*	36,006 ± 22,972
	Symptomatic	432 ± 464*	37,043 ± 22,762
Spike-specific IgA (A.U./ml) (mean ± standard deviation)[h]	Total	2.96 ± 1.94*	8.37 ± 0.88
	Asymptomatic	2.58 ± 1.39*	8.41 ± 0.89
	Symptomatic	3.57 ± 2.59*	8.32 ± 0.88
NP-specific T cells (SFC) (mean ± standard deviation)[f]	Total	14 ± 7.5*	18 ± 16.0
	Asymptomatic	14 ± 4.1	19 ± 12.3
	Symptomatic	14 ± 9.6	18 ± 19.0
NP-specific IgG (A.U./ml) (mean ± standard deviation)[g]	Total	3.5 ± 2.5	3.6 ± 2.4
	Asymptomatic	5.2 ± 3.0	5.0 ± 2.7
	Symptomatic	2.5 ± 1.6	2.6 ± 1.6

*$p < 0.05$, comparing T1 vs. T2, using McNemar test or paired-sample *t*-test; no differences were found for symptomatic vs. asymptomatic individuals using *t*-test; [a] borderline/equivocal T-Spot result: 11 out of 50 individuals; non-reactive: 8 out of 50; [b] borderline/equivocal T-Spot result in 1 out of 49 individuals; [c] individuals with reactivity for SARS-CoV-2, either by the presence of SARS-CoV-2-specific T cells (by T-Spot) or IgG (by chemiluminescent microparticle immunoassay); [d] borderline/equivocal T-Spot result: 1 out of 50 individuals; non-reactive: 17 out of 50; [e] borderline/equivocal T-Spot result in 3 out of 49 individuals; non-reactive: 16 out of 49; [f] measured as the number of spot forming cells (SFC) per 250,000 PBMCs in the T-Spot assay, and considering only the individuals reactive for T-Spot; [g] level of serum IgG, considering only the individuals reactive for IgG; [h] level of serum IgA, considering only the individuals reactive for IgA; T1, immediately before the administration of the vaccine; T2, 15 days after the administration of the vaccine; NP, Nucleocapside Protein; SFC, spot forming cell; A.U., arbitrary units

cells. Notwithstanding, we may not overlook the possibility of SARS-CoV-2-specific memory T cells being localized in other tissues rather than in the peripheral blood [4, 20, 21]. According to our results, it seems that some individuals are strong responders and still reactive for Spike-specific T cells 6 months after infection, while others are weak responders (non-reactive 6 months post infection). The strong responders develop a *T* cell response of greater magnitude upon vaccination (102 ± 82 SFC) compared to weak responders (39 ± 23 SFC). Despite that, a single dose of COVID-19 vaccine is enough for the development of Spike-specific T cells in these weak responders, whose magnitude of the response is similar to that obtained in infection-naive individuals after two doses of COVID-19 mRNA vaccine [manuscript in preparation].

Our results also point out that a robust cellular response to SARS-CoV-2 is linked to a strong humoral response. Thus, the monitoring of the cellular and humoral response could allow the identification of individuals with a weaker SARS-CoV-2-specific immune response, and that would benefit from additional vaccine doses. In this sense, we further studied circulating $CD4^+$ Tfh cells, and their kinetics upon vaccination, as these cells link cellular and humoral immunity.

A single dose of vaccine restored Spike-specific T cells, IgG and IgA antibodies

Fifteen days after the vaccine administration (T2), there was a significant rise in the percentage of recovered participants reactive for Spike-specific T cells (from 64 to 98%), IgG (from 90 to 100%) and IgA (from 48 to 98%). Noteworthy, our group obtained similar percentages for Spike-specific T cells and IgG after two doses of the vaccine in infection-naive individuals [manuscript in preparation]. In the same line, the levels of Spike-specific T cells (measured as the number of spot forming cells (SFC) per 250,000 PBMCs) and IgG increased sharply upon a single dose of vaccine in recovered participants

(Table 1), reaching values markedly higher than those obtained by our group for infection-naive individuals, after two doses of the vaccine [manuscript in preparation], which is in agreement with the results previously reported [22, 23]. These results demonstrated that a single dose of COVID-19 vaccine efficiently stimulated both arms of the adaptive immunity, which is in accordance with previous studies on Pfizer-BioNTech BNT162b2 mRNA vaccine [24, 25]. A single dose of vaccine is also suffice to restore anti-Spike IgA antibodies, whose levels decrease 2 months after infection [26] and, 6 months after infection, only 48% of the recovered individuals maintain Spike-specific IgA antibodies, as demonstrated here. Importantly, a single dose of COVID-19 vaccine was also demonstrated to trigger a humoral and cellular immune response in patients receiving immunosuppression [27]. Nevertheless, it is mandatory a longitudinal study to find out how long vaccine-induced T cells and antibodies persist, and whether additional doses of the vaccine are recommended.

No differences were found between symptomatic and asymptomatic individuals

No differences were found when comparing symptomatic and asymptomatic recovered individuals, either at T1 or T2, in what concerns to IgA Spike-specific antibodies serum levels, IgG Spike- and NP-specific antibodies serum levels, Spike- and NP-specific T cells (Table 1), or the percentage and absolute number of circulating $CD4^+$ T follicular helper (Tfh) cells and their subpopulations. However, some studies reported an increase of IgG levels and of some Tfh cell subsets in severe COVID-19, when compared to non-severe disease [9, 10], but the time since recovery is shorter than the one in our study and, as described by Zhao et al., the altered parameters tend to normalize with time [13].

COVID-19 vaccine mobilizes $CD4^+$ T follicular helper cells and bias their polarization toward a Th17 profile

According to our results, recovered participants (at T1) displayed profound alterations in the proportion of circulating Tfh cells, as well as in their activation status, functional compartment distribution and polarization, even 6 months after SARS-CoV-2 infection onset, when compared to the basal levels evaluated in naive-infection donors. The changes in the numbers and function of Tfh cells observed here, and by others during the course of SARS-CoV-2 infection, may justify the impaired development of an effective humoral response in this disease [9, 10, 13, 15]. Still, it is important to emphasize that our study demonstrates that some changes in Tfh subsets persists at least until 6 months after the infection onset; and to conjecture about how those alterations challenge an effective immune response in these individuals and whether they are reverted upon vaccination. Our recovered participants presented a significant decrease of the absolute number and percentage of circulating Tfh cells measured in whole blood (99 ± 51 cells/µl and $1.32\% \pm 0.55$) or within $CD4^+$ T cells ($12\% \pm 3.02$), compared to infection-naive donors (155 ± 54 cells/µl, $2.34\% \pm 1.04$ and $17\% \pm 4.79$, respectively). In the same line, the absolute number and percentage of activated $CD25^+$ Tfh (17 ± 14 cells/µl and $17\% \pm 8.22$) and HLA-DR^+ Tfh cells (2.65 ± 1.74 cells/µl and $2.86\% \pm 1.58$) were significantly decreased in recovered participants, compared to infection-naive donors (77 ± 32 cells/µl and $43\% \pm 8.65$, for $CD25^+$ Tfh; 6.15 ± 4.36 cells/µl and $3.80\% \pm 1.59$, for HLA-DR^+ Tfh), as depicted in Fig. 2a and Supplementary Information Table 1, Online Resource 1. The proportion of Tfh cells in the central memory (CM) compartment is higher in recovered participants, however, according to our results, these cells are less prone to undergo Tfh17 differentiation than CM Tfh cells from infection-naive donors, as we observed $39\% \pm 8.90$ (44 ± 10 cells/µl) in this latter group, whereas recovered participants displayed a percentage of $24\% \pm 9.0$ (22 ± 15 cells/µl) CM Tfh17 cells. In the same line, there is a decrease in CM Tfh cells from recovered participants polarized toward Tfh1 and Tfh1/17 (Fig. 2b and 2c; Supplementary Information Table 1, Online Resource 1). CM Tfh1 reduction has been previously described in the course of the infection or shortly after recovering, but, in opposition to our results, some studies reported and increased percentage of CM Tfh17 in during severe COVID-19, as well as 4–9 weeks after recovering, and no differences 20–26 weeks after recovering [13, 28, 29]. Our data point out important long-term effects of SARS-CoV-2 infection in Tfh cells, translated as a marked decrease in Tfh cell numbers and activation status, as well as an altered polarization pattern, especially affecting Tfh17 cells.

It has been previously reported that $CD4^+$ T cells from COVID-19 patients showed impaired ability to undergo differentiation into Tfh cells, leading to reduced numbers of this cell subset in the secondary lymphoid organs, and impairing germinal centers formation [15]. This decrease is observed at peripheral level in our study, 6 months after the infection onset. Conversely, an increased proportion of cytotoxic $CD4^+$ Tfh cells is described in the course of SARS-CoV-2 infection, being hypothesized that this cell subset kills B cells, and hampers the development of the humoral response [10]. In this context, a vaccine able to properly mobilize and activate Tfh cells would overcome this flaw.

In-line with this, when comparing recovered participants before (T1) and 15 after the vaccine administration (T2), our results showed an increase in the percentage Tfh cells (within $CD4^+$ T cells) in the PB, from $12\% \pm 3.02$ at T1, to $14\% \pm 3.42$ at T2 (Fig. 2a; Supplementary Information Table 1, Online Resource 1), which is in accordance with a previous study reporting that mRNA vaccine increases circulating Tfh cells in both recovered and infection-naive individuals

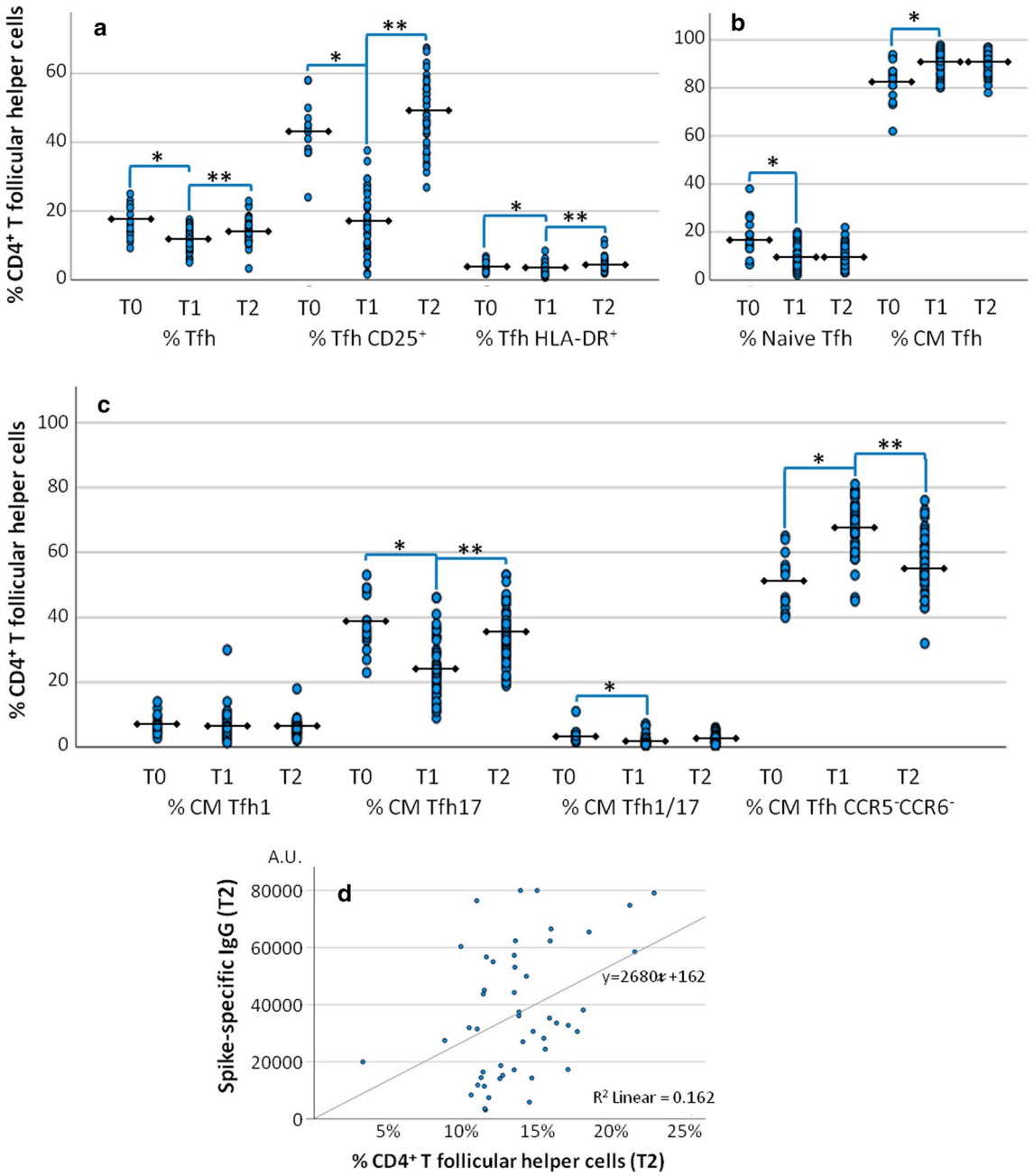

Fig. 2 Frequency of circulating Tfh cells and their subsets, immediately before (T1) and 15 days after (T2) the vaccine administration to individuals recovered from SARS-CoV-2 infection (**a–c**); T0 corresponds to the basal levels of circulating Tfh cells and their subsets, evaluated in an age- and gender-matched control group of infection-naive donors (**a–c**); correlation between the frequency of circulating Tfh cells and serum levels of Spike-specific IgG at T2 (**d**); the horizontal line represents the average; CM Tfh, central memory $CD4^+$ T follicular helper cells; Tfh1 corresponds to Th1-polarized Tfh cells, identified as $CD3^+CD4^+CD8^-CXCR5^+CCR5^+CCR6^-$; Tfh17 corresponds to Th17-polarized Tfh cells, with the following phenotype: $CD3^+CD4^+CD8^-CXCR5^+CCR5^-CCR6^+$; Tfh1/17 corresponds to Th1/17-polarized Tfh cells, identified as $CD3^+CD4^+CD8^-CXCR5^+CCR5^+CCR6^+$; Tfh $CCR5^-CCR6^-$ corresponds to Tfh cells non-polarized toward Th1 nor Th17 phenotype; R^2 Linear = 0.162 corresponds to the coefficient of determination of the linear regression; $^*p < 0.05$ for T0 vs. T1, using the Mann–Whitney test; $^{**}p < 0.05$ for T1 vs. T2, using the paired-sample *t*-test

[30]. The proportion of activated Tfh cells circulating in the PB also raised upon vaccination, from 16.6% ± 8.22 (T1) to 48.5% ± 9.95 (T2) for Tfh $CD25^+$ cells, and from 2.86% ± 1.58 (T1) to 4.69% ± 1.83 (T2) for Tfh HLA-DR^+ cells (Fig. 2a; Supplementary Information Table 1, Online Resource 1). In fact, this increase was also verified in the absolute numbers of circulating Tfh $CD25^+$ (17 ± 14 at T1, and 49 ± 19 cells/μl at T2) and Tfh HLA-DR^+ cells, raising from 2.65 ± 1.74 at T1 to

4.58 ± 2.07 cells/µl at T2. Additional data on Tfh subsets percentages and absolute count values are given in Supplementary Information Table 1, Online Resource 1. No differences were found in the distribution of Tfh cells among naive and central memory (CM) compartments between T1 and T2 (Fig. 2b, Supplementary Information Table 1, Online Resource 1). Nonetheless, vaccination polarized CM Tfh cells toward Th17 phenotype, as the percentage of CM Tfh17 (i.e., $CCR5^{-}CCR6^{+}$ CM Tfh cells) raised from 24.1% ± 9.0 (T1) to 34.9% ± 8.1 (T2) (Fig. 2c, Supplementary Information Table 1, Online Resource 1). According to the data published so far, Tfh17 cells play an important role in the development of an IgA response [31], which can constitute an effective protection against the infection of airway epithelial cells by SARS-Cov-2. Importantly, our study demonstrated that the reduced levels of circulating Tfh17 cells, observed 6 months after SARS-CoV-2 infection, can be restored with a single dose of Pfizer-BioNTech BNT162b2 mRNA COVID-19 vaccine. Noteworthy, this augment of Tfh17 cells was accompanied by a striking increase in the percentage of individuals reactive for Spike-specific IgA (from 48% at T1 to 98% at T2), as well as Spike-specific IgA titers (Table 1). Our results demonstrate that vaccination mobilizes and activates Tfh cells, probably allowing a proper function of this cell subset, leading to an effective development of the humoral response against the virus.

The percentage of circulating $CD4^{+}$ T follicular helper cells correlates with Spike-specific IgG levels

After the administration of the vaccine (T2), it was observed a positive correlation between the percentage of circulating Tfh cells and the serum levels of IgG Spike-specific antibodies (Pearson's correlation coefficient: $r = 0.403$; $p = 0.04$), as depicted in the Fig. 2d. A similar correlation was previously described for convalescent individuals [9]. Of note, despite the positive correlation between circulating Tfh cells and Spike-specific IgG serum levels described here and by others [9], the coefficient of determination of the linear regression (R^2 Linear = 0.162) suggests our regression model has a very limited predictive value.

Conclusion

This study demonstrates that a single dose of Pfizer-BioNTech mRNA COVID-19 vaccine efficiently restores Spike-specific IgG and IgA antibodies and T cells, in individuals recovered from SARS-CoV-2 infection, 15 days after administration. According to our data, the vaccination mobilizes and activates Tfh cells, suggesting that the vaccine elicits a functional Tfh response. This is of utmost importance as SARS-CoV-2 infection hinders Tfh differentiation and function, preventing a proper development of the humoral response. Our data show that vaccination restores the number of circulating Tfh cells and their ability to undergo Tfh17 polarization, which corroborates the thesis that vaccination can restore the functional Tfh response lost in the course of SARS-CoV-2 infection and, subsequently, promote an efficient humoral response; therefore, vaccinated individuals can achieve a higher level of immune protection against SARS-CoV-2. Accordingly, we found a positive and significant correlation between Tfh cells and IgG levels, 15 days after the vaccine administration.

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Declarations

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Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of the Centro Hospitalar e Universitário de Coimbra (CHUC) (OBS.SF.107–2021).

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