Heliyon 8 (2022) e09863

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Robust SARS-COV-2-specific T-cell immune memory persists long-term in immunocompetent individuals post BNT162b2 double shot

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ARTICLE INFO

Keywords:
COVID-19
T cell immune response
SARS-CoV-2
Humoral immune response
SARS-CoV-2-specific T cells
Ex vivo model

ABSTRACT

Background: A robust efficiency of mRNA vaccines against coronavirus disease-2019 has been demonstrated, however, the intended long-term protection against SARS-CoV-2 has been challenged by the waning humoral and cellular immunity over time, leading to a third vaccination dose recommendation for immunocompetent individuals, six months after completion of primary mRNA vaccination.

Methods: We here measured humoral responses via an immunoassay measuring SARS-CoV-2 neutralizing antibodies and T-cell responses using Elispot for interferon-γ 1- and 8- months post full BNT162b2 vaccination, in 10 health-care professionals. To explore whether the declining abundance of coronavirus-specific T-cells (CoV-2-STs) truly reflects decreased capacity for viral control, rather than the attenuating viral stimulus over time, we modeled ex vivo the T-cellular response upon viral challenge in fully vaccinated immunocompetent individuals, 1- and 8-months post BNT162b2.

Findings.: Notwithstanding the declining CoV-2-neutralizing antibodies and CoV-2-STs, re-challenged CoV-2-STs, 1- and 8-months post vaccination, presented similar functional characteristics including high cytotoxicity against both the unmutated virus and the delta variant.

Interpretation.: These findings suggest robust and sustained cellular immune response upon SARS-CoV-2 antigen exposure, 8 months post mRNA vaccination, despite declining CoV-2-STs over time in the presence of an attenuating viral stimulus.

1. Introduction

Efficacy of COVID-19 vaccines in protecting against severe disease development, hospitalization and mortality has been demonstrated both in clinical trials and retrospective studies [1]. However, as we approach the 1-year mark of initial COVID-19 vaccine rollout there have been increasing concerns over the long-term vaccination protection, resulting in the implementation of 3rd booster vaccine strategies for certain risk populations and recommendation for a 3rd dose in general population as well. However, data on the durability of protection post SARS-CoV-2 vaccination are limited, thus hindering the decision making process on the necessity and the timing of a booster vaccination for immunocompetent individuals.

Protective immunity for Ad26.COV2.S (Johnson & Johnson–Janssen) vaccine has been reported to last for at least 8 months after the single-shot vaccine regimen or 6 months after the two-shot vaccine regimen, in 10 and 10 participants, respectively [2]. Regarding mRNA vaccines (mRNA-1273 manufactured by Moderna and BNT162b2 by Pfizer–BioNTech), humoral responses have shown waning antibody kinetics from peak levels, six months after the second dose [3, 4, 5, 6], whereas a continued increase in functional memory B cells has been documented in SARS-CoV-2-naïve vaccinees [5]. In contrast, T cell response kinetics have been relatively understudied; typical T responses are characterized by an effector phase reaching peak immunity levels 3-months post vaccination and a following contraction phase with decreasing or stabilizing T-cell immunity up to 8 months post-vaccination [5, 6, 7].

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https://doi.org/10.1016/j.heliyon.2022.e09863
Received 7 January 2022; Received in revised form 23 April 2022; Accepted 29 June 2022

Real-world evidence showed reduced effectiveness of BNT162b2 vaccination against SARS-CoV-2 infection over time, however, BNT162b2 significantly prevented hospital admissions up until 6 months after full vaccination, in a large cohort of 3,436,957 individuals working in an integrated health-care organization [1].

Existing data on T-cell immune memory post vaccination are simply based on the assessment of cytokine secretion upon a short stimulation with SARS-CoV-2 peptides, inadequately simulating the cell-mediated immune response upon virus challenge, leaving it unclear how effector T cells would respond at a much later exposure to virus since the time of vaccination. We here, investigated over time, the development of the two components of antigen-specific immune memory against SARS-CoV-2 after BNT162b2 vaccination; the humoral (in terms of neutralizing antibody viral inhibition) and cellular [in terms of functional spike-specific T cells (spike-STs)] immunity in ten fully vaccinated, SARS-CoV-2-naïve, health-care professionals, at one- and 8-months post BNT162b2 vaccination. Moreover, by re-challenging and expanding spike-STs, we modeled ex vivo the functionality of spike-STs, simulating an in vivo condition of increasing virus load. Spike-STs were tested against the parental WA1/2020 strain as well as the most common SARS-CoV-2 variants of concern, the B.1.1.7 (alpha), B.1.351 (beta) and B.1.617.2 (delta) variant.

2. Methods

2.1. Study population

We enrolled ten fully vaccinated health-care professionals without prior SARS-CoV-2 infection one- and 8-months post the first dose of BNT162b2 vaccination after written informed consent. The study was approved by the Institutional Review Board of the George Papanikolaou hospital and was conducted in accordance with the Helsinki Declaration.

2.2. Humoral responses

After vein puncture, sera were collected on months one and eight post BNT162b2 vaccination. Serum was separated within 4 h from blood collection and stored at −80 °C. Inhibition by neutralizing antibodies against SARS-CoV-2 (CoV-2-NAbs) were measured using an FDA approved methodology for diagnostic use (cPass™ SARS-CoV-2 NAbs Detection Kit; GenScript, Piscataway, NJ, USA). Inhibition of at least 30% is considered positive, whereas >50% has been associated with clinically relevant viral inhibition.

2.3. T cell responses

T cell responses were studied as previously described [8]. PBMCs were pulsed with overlapping peptides of the full length spike protein (jpt peptide Technologies) and the secretion of interferon-gamma (IFN-γ) was measured by Elispot. Spot-forming cells (SFCs) were counted on Eli.Scan Elispot scanner (A.EL.VIS) using Eli.Analyse software V6.2.SFC. SARS-CoV-2 spike-specific T cells (spike-STs) were expressed as SFCs per input cells. Response was considered positive if the total cytokine–producing SFCs against spike, were ≥30 per 5×10^5 PBMCs.

2.4. CoV-2-ST re-challenge and expansion

PBMCs, derived from 15-20ml of blood were pulsed for 30min with 0.5 μg/ml of the full length spike pepmixes (jpt peptide technologies) and cultured in G-Rex10 bioreactors (Wilson Wolf) in media supplemented with 10 ng/ml interleukin-7 (IL-7) and 400U/ml IL-4 until day 9–11, as previously described [9, 10, 11, 12].

2.5. Phytohaemagglutinin (PHA) blast generation

PHA blasts were generated from PBMCs by using PHA and maintaining the cells in T-cell media supplemented with IL-2 (100 U/ml), replenished every 3 days.

2.6. Immunophenotyping

CoV-2-STs were stained with CD45, CD3, CD4, CD8, CD45RA, CCR7, CD27, CD28, programmed death-1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and T-cell immunoglobulin and mucin domain-containing protein 3 (TIM3). T-cell subsets were defined as follows: naive; $CD45RA^+$ $CCR7^+$, effector memory (EM); $CD45RA^-$ $CCR7^-$, central memory (CM); $CD45RA^-$ $CCR7^+$ and TEMRA; $CD45RA^+$ $CCR7^-$.

2.7. Cytotoxicity

Autologous non-pulsed PHA blasts were labeled with a low concentration of CFSE (0.625μM), while autologous, 2h-antigen-pulsed PHA blasts were labelled with high concentration of CFSE (5μM). Both populations were mixed (1:1) and co-cultured with CoV-2-STs at various effector (E) to target (T) ratios in triplicates. After 20h-incubation, dead cells were stained with 7AAD and the percentage of specific cytotoxicity was calculated as follows:

$$\text{Cell lysis (\%)} = 100\text{–}[100*\text{Sample}(CFSE^{high}/CFSE^{low})/\ \text{Baseline}(CFSE^{high}/CFSE^{low})]$$

All FCM data were analyzed in parallel in a FACS Calibur with the CellQuest Pro6 software (Becton Dickinson).

2.8. Statistical analysis

Data were analyzed using R (v 4.1.1). Descriptive statistics were performed using median (interquartile range) or mean ± standard error for continuous variables according to normality, and frequency for categorical variables. Continuous variables were compared using paired Student's t-test. Effects of time from vaccination and age on number of CoV-2-STs and CoV-2-NAbs were determined by fitting a linear regression model. Correlation between continuous variables was assessed with the Pearson's correlation coefficient. P-values ≤0.05 were considered significant.

3. Results

Ten immunocompetent, previously unexposed health-care professionals (six women and four men) of median age 55 (range: 29–67) years, who received BNT162b2 vaccination were prospectively enrolled in the study (Figure 1A). Blood samples were drawn after written informed consent at a median of 31 [interquartile range (IQR = 2)] and 245 (IQR = 4) days after first vaccination dose.

Protective levels of circulating CoV-2-NAbs were detected in all vaccinees by an enzyme-linked immunosorbent assay, one-month post vaccination [median inhibitory activity 95.2 (IQR = 1.3)], but decreased by a factor of 2.3 at month 8 [41.7 (IQR = 26.1); $p < 0.0001$; Figure 1B). Only 3/10 vaccinees maintained highly protective humoral immunity (>50% viral inhibition), 3/10 had detectable (>30% < 50%) CoV-2-NAbs and 4/10 lost humoral immunity, 8-months post vaccination.

T cell responses against SARS-CoV-2 spike antigen were measured as interferon-γ secreting cells by Enzyme-Linked Immunospot Assay after pulsing peripheral blood mononuclear cells (PBMCs) with spike pepmixes. Circulating SARS-CoV-2–specific T cells (CoV-2–STs), 8 months post vaccination, were present, albeit significantly less by a factor of 2.6 over the first month, in all 10 vaccinees, [median (IQR) spot forming cells (SFCs)/5×10^5 PBMCs on month 1 and 8: 656 (264) vs 257 (203), respectively; $p = 0.002$; Figure 1C). There was a good correlation between the kinetics of CoV-2-NAbs and spike-STs (Pearson $r = 0.802$; $p < 0.0001$) while age was negatively correlated with T-cellular and humoral responses, yet statistically significant only for T-cellular responses (Pearson r = -0.73, t-statistic p-value = 0.0167 and r = -0.62, t-statistic p-value = 0.0588, respectively; Figure 1D).

The magnitude of antigen-specific T cell response however, coincides with either cell expansion or contraction kinetics, depending on antigen encounter at the time of testing [13]. To investigate the real dynamics of T

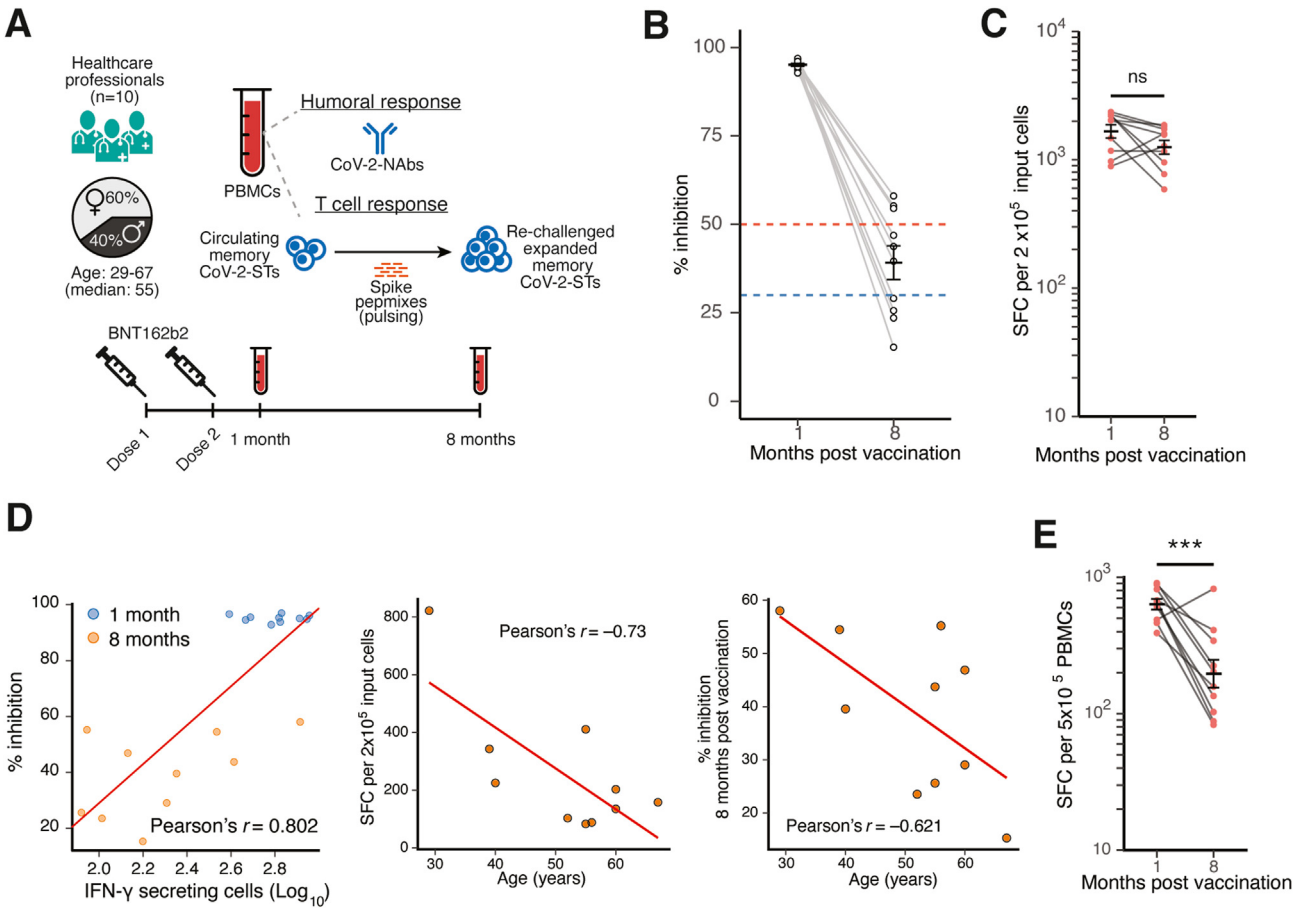

Figure 1. Humoral and T cell immune responses after BNT162b2 vaccination. Panel A graphical outline of study design. Panel B Quantification of neutralizing antibodies against SARS-CoV-2 (CoV-2-NAbs) at 1- and 8-months post vaccination as % inhibition of the interaction between the receptor binding domain (RBD) of the viral spike glycoprotein with the ACE2 cell surface receptor. Panel C Circulating coronavirus-2-specific T cells (CoV-2-STs) per 5×10^5 peripheral blood mononuclear cells (PBMCs) on month 1 and 8 post vaccination. Panel D Scatterplots of CoV-2-NAbs versus CoV-2-STs at both timepoints tested (upper panel), circulating CoV-2-STs against donor age (middle panel) and CoV-2-NAbs against donor age (lower panel). Panel E Ex vivo expanded CoV-2-STs per 2×10^5 input cells on month 1 and 8 post vaccination.

cell responses and elucidate whether the lower CoV-2-ST numbers at a later time-point truly reflect an attenuated T cell immunity, we mimicked the natural infection by re-challenging same donor PBMCs with spike overlapping peptide libraries and expanding them in vitro for 10 days. Importantly, and similar to what we have previously shown in COVID-19 convalescents [8], the re-challenged, same donor-generated CoV-2-STs, presented similar and robust anti-spike specificity, both at 1 and 8 months post vaccination [median (IQR) SFCs/2×10^5 input cells at month 1 and 8: 2,030 (982) vs 1,424 (694), respectively; p = ns; Figure 1E].

To further functionally characterize the re-challenged spike-STs generated early or later post vaccination, a cytotoxicity assay was performed using one- and 8-month re-challenged spike-STs from all ten donors, against autologous spike-pulsed phytohaemagglutinin (PHA) blasts. At both time points post vaccination, the re-challenged CoV-2-STs induced strong, specific, and comparable lysis of antigen-pulsed PHA blasts [median (IQR)% lysis, 40:1 ratio, month 1 and 8: 57 (39.2) vs 67 (17.8), respectively; p = ns; Figure 2A). These cytotoxicity data, along with the observed high specificity of the expanded CoV-2-STs, strongly suggest that memory spike-STs, either at 1- or 8-months post vaccination, can be rapidly activated in vivo upon antigen encounter and be similarly protective against the virus. Importantly, vaccinated donor-CoV-2-STs, 8 months post vaccination, could provide cross-protection against all current SARS-CoV-2 variants of concern, including the alpha, beta, and delta variants (p = ns; Figure 2B), thus suggesting long-lasting T cell protective immunity post vaccination against all common mutations. We have previously shown that vaccinated donor-CoV-2-STs are highly cytotoxic against alpha and beta mutants [8] and here, we demonstrate effective lysis of delta strain, 8 months post vaccination, equal to the corresponding lysis of unmutated virus [median (IQR) lysis at 40:1 ratio, unmutated: 67 (17.8) vs delta strain: 62 (15.1); p = ns; Figure 2C).

In terms of immunophenotype, at both time points, the ex vivo expanded CoV-2-STs were mainly CD4+ cells but also CD8+ T-cells, predominantly expressing central and effector memory markers and having an activated and non-exhausted profile (Figure 2D).

4. Discussion

Studies on immune responses post COVID-19 have shown humoral and T cell immunity persisting for at least 8 months. However, the robustness of immune memory post vaccination remains rather poorly understood [14]. We herein provide insight into the durability and functionality of immunological responses of healthy immunocompetent individuals, following mRNA BNT162b2 vaccination. We show that a significant waning of humoral and SARS-CoV-2-specific T-cell responses occurs within 8 months post inoculation, negatively correlating with age. However, despite the shrinkage of the reservoir of CoV-2-STs 8 months post mRNA vaccination, these predominantly memory cells, are able to expand ex vivo if re-challenged and presumably provide protection not only against the unmutated SARS-CoV-2, but also against the currently problematic delta variant.

Given the importance of the long-lasting immunity in the battle against the pandemic, several groups have studied the robustness of

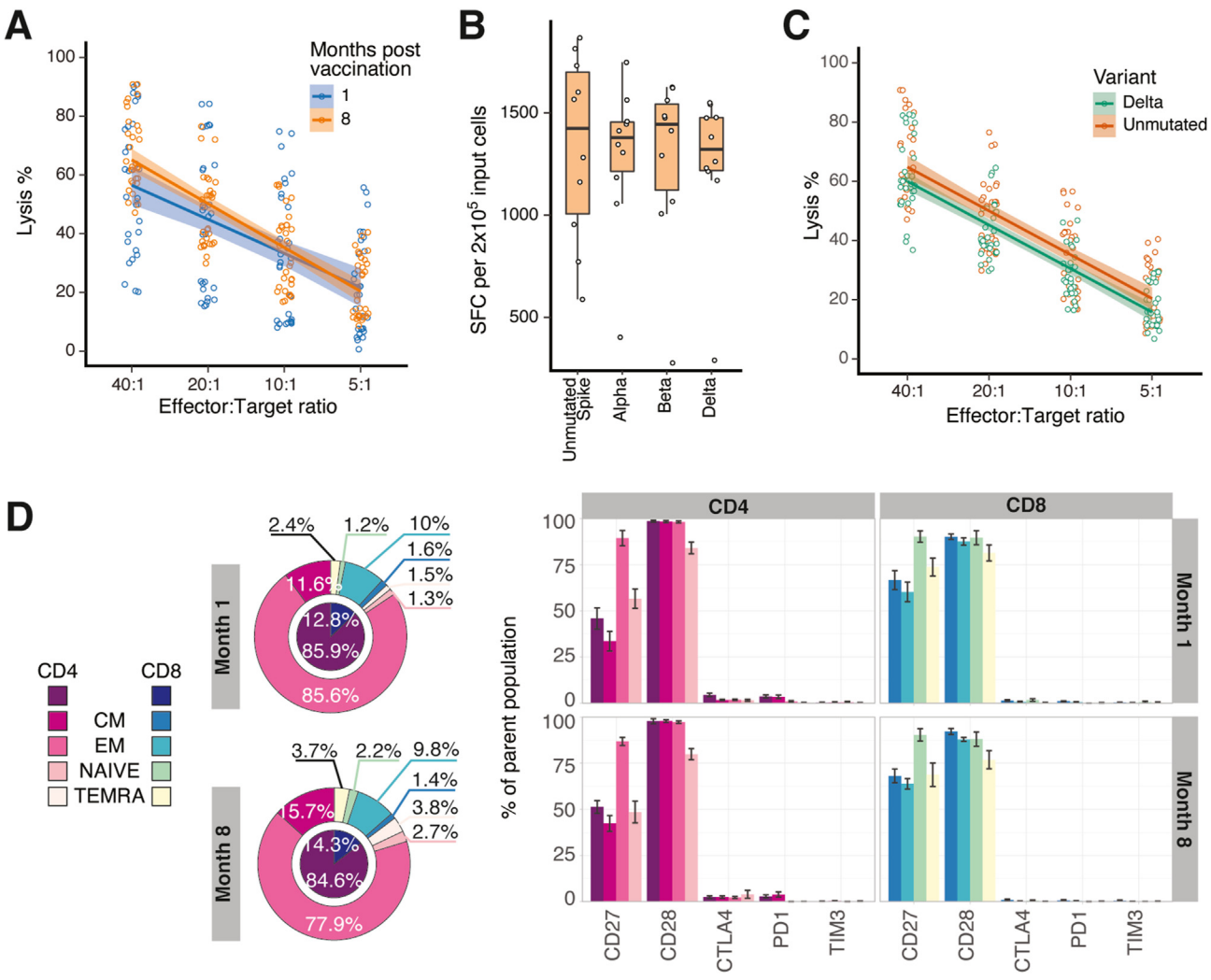

Figure 2. Phenotypic and functional characteristics of spike re-challenged and ex vivo expanded CoV-2-STs from BNT162b2 vaccinees. Panel A Cytotoxic activity of CoV-2-STs expanded 1-month (blue dots) and 8-months (orange dots) post vaccination against autologous, unmutated spike-pulsed phytohaemagglutinin (PHA) blasts. Regression lines per group and ± SE ribbons are shown. Panel B Number of interferon-γ secreting CoV-2-STs expanded 8-months post vaccination after pulsing with the unmutated SARS-CoV-2 spike antigen, B.1.1.7 (alpha), B.1.351 (beta) and B.1.617.2 (delta) SARS-CoV-2 variants. Boxplots denote median and quartile bounds. Whiskers are placed at 1.5x interquartile range. Panel C Cytotoxic activity of CoV-2-STs expanded 8-months post vaccination against autologous, PHA blasts pulsed with the unmutated spike antigen (orange dots) or the delta variant (green dots). Regression lines per group and ± SE ribbons are shown. Panel D Immunophenotypic profile of CoV-2-STs expanded 1-month and 8-months post vaccination. CM: central memory; EM: effector memory.

immune responses in COVID-19 vaccinees. Studies reported up to now have mainly focused on the longevity of humoral responses showing CoV-2-NAbs decay over time [3, 4, 15] thus challenging the notion that this branch of immunity serves as the main provider of long-term protection against SARS CoV-2. We here observed a significant age-dependent decrease of CoV-2-NAbs during the 8-month follow-up, contrasting the very strong neutralizing activity in all individuals one-month post vaccination (CoV-2-NAbs >92.8%). Only 3 of 10 (30%) vaccinees maintained clinically relevant viral inhibition (>50%) 8 months post mRNA vaccination.

The T-cell mediated branch of the immune system is critical for both short and long-term protection, however the dynamics of these memory cells remain poorly understood. We [16] and others [17] have shown that immunocompromised individuals under active immunosuppression, fail to mount protective humoral and T cell specific responses after complete mRNA vaccination, remaining vulnerable to COVID-19 infection. For the immunocompetent subjects however, the duration and robustness of T-specific immune response to vaccination is still exploratory.

Herein, in agreement with Collier et al [7], we report that T-cell responses remain present at least 8 months post BNT162b2 vaccine, extending the findings of recent reports on 3–6 months T-cell durability post mRNA vaccines [5, 6] and showing comparable longevity with the immune memory induced by the Ad26.COV2.S vaccine [2]. Nevertheless, while a memory reservoir of CoV-2-STs and CoV-2-NAbs remained detectable 8 months post vaccination, there was a significant reduction by a factor of 2.6 and 2.3, respectively, correlating with increasing age. So far, studies on the persistence of specific T-cell memory post vaccination were solely focusing on the quantification of CoV-2-STs. Here, we sought to explore whether this reduction simply reflects the attenuation of the antigenic stimulus late post vaccination rather than inability of CoV-2-STs to expand and mount a robust immune response against an in vivo exposure to virus. Indeed, we have previously shown in COVID-19 patients that the T-cell immunity status is not reliably represented by the CoV-2-ST's levels *per se*; instead, it is the magnitude of CoV-2-ST's in vivo expansion that can discriminate patients able to self-control the infection from those having high probability to severely deteriorate [8].

On this basis, we interrogated and compared the characteristics of spike re-challenged and ex vivo expanded CoV-2-STs from 10 immunocompetent donors at one- and 8-months post vaccination, in order to model ex vivo the ability of memory T cells to expand and provide

protection against COVID-19. Importantly, same donor-derived CoV-2-STs, one- (early) and 8-months (late) post vaccination, presented similar trajectories after encountering the spike stimulus despite marked decrease in their absolute numbers over time. In particular, ex vivo expanded, both late or early re-challenged CoV-2-STs, were qualitatively similar, sharing phenotypical and functional attributes and presenting an activated, non-exhausted, and predominantly memory profile. Notwithstanding that phenotype may have been altered in these ex-vivo expanded cells post stimulation and expansion in culture, it is well recognised that a similar memory profile of other ex vivo expanded and clinically tested virus-specific T cells has been associated with efficacy in clinical trials [10, 18]. Their postulated activity in vivo, was further supported by the observation that both, early and late re-challenged CoV-2-STs were able to efficiently lyse spike-pulsed target cells suggesting strong cytotoxic function at later time points and major contribution to future recall responses.

Current and future emerging SARS-CoV-2 variants of concern could potentially jeopardize the effectiveness of vaccines in curbing the pandemic, if they allow the virus to partially evade vaccine-induced immune responses. Reduced neutralization of emerging variants by CoV-2-NAbs was reported after 3–8 months post mRNA vaccination [7, 19, 20]. Unlike CoV-2-NAbs, CoV-2-STs have been shown to be less affected by viral mutations, as they can recognize a plethora of viral epitopes of the spike protein [21, 22, 23], secreting interferon-γ upon recognition of SARS-CoV-2 mutants. Here, we show that memory CoV-2-STs persisting 8-months post BNT162b2 vaccination cross-react with alpha, beta, and delta strains and efficiently lyse the currently predominant delta strain-pulsed target cells, an effect of utmost importance for the long-term shielding from current and future SARS-CoV-2 variants of concern.

The health-care professionals used as donors in this study, although at the front line of the coronavirus pandemic, represent a small sample without comorbidities and as such, a potential caveat of the study. Large cohort studies including longitudinal sampling of donors, immune monitoring and monitoring of vaccine effectiveness against infection or severe disease, will identify correlates of clinical protection and unravel the relevance of long-term SARS-CoV-2 immune memory.

Overall, our data provide evidence of persistent, broad repertoire, and functional CoV-2-specific T–cell responses in healthy individuals for at least 8 months post BNT162b2 vaccine, highlighting the major contribution of T-cell immunity to long-term protection and importance of wide-spread vaccination to shield people against SARS-CoV-2 and its variants. These observations may facilitate the policy making on the timing of the administration of a third booster vaccination in immunocompetent individuals.

Declarations

Author contribution statement

Conceived and designed the experiments, Anastasia Papadopoulou, Achilles Anagnostopoulos and Evangelia Yannaki.

Performed the experiments, Fani Stavridou, Maria Giannaki, Kiriaki Paschoudi, Fani Chatzopoulou and Eleni Gavriilaki.

Analyzed and interpreted the data, Anastasia Papadopoulou, Fani Stavridou, Maria Giannaki, Kiriaki Paschoudi, Fani Chatzopoulou, Grigorios Georgolopoulos and Evangelia Yannaki.

Contributed reagents, materials, analysis tools or data, Achilles Anagnostopoulos and Evangelia Yannaki.

Wrote the article, Anastasia Papadopoulou, Grigorios Georgolopoulos and Evangelia Yannaki.

Funding statement

This work was supported by the Greece 2021 Committee (ΑΔΑ: 49 6ΔΠΡ46906Β-8ΝΕ).

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

Funding for this project was provided by Greece 2021 Committee (ΑΔΑ: 6ΔΠΡ46906Β-8ΝΕ).

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