Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Infection 84 (2022) 171–178

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

SARS-CoV-2 antibody and T cell responses one year after COVID-19 and the booster effect of vaccination: A prospective cohort study

Willem A. Mak[a], Johannes G.M. Koeleman[a], Marijke van der Vliet[a], Frans Keuren[a], David S.Y. Ong[a,b,*]

[a] Department of Medical Microbiology and Infection Control, Franciscus Gasthuis and Vlietland, Kleiweg 500, Rotterdam 3045 PM, the Netherlands
[b] Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Universiteitsweg 100, Utrecht 3584 GC, the Netherlands

ARTICLE INFO

Article history:
Accepted 7 December 2021
Available online 9 December 2021

Keywords:
COVID-19
SARS-CoV-2
Immunity
Vaccination
T cell
Antibody

SUMMARY

Objectives: First, to describe SARS-CoV-2 T cell and antibody responses in a prospective cohort of healthcare workers that suffered from mild to moderate COVID-19 approximately one year ago. Second, to assess COVID-19 vaccine-induced immune responses in these prior-infected individuals.
Methods: SARS-CoV-2-specific T cell and anti-SARS-CoV-2-Spike-RBD immunoglobulin G (IgG) responses in blood were determined before COVID-19 vaccination with mRNA-1273, BNT162b2, Ad26.CoV2-S or ChAdOx1-S, two weeks after first vaccination, and after second vaccination.
Results: 55 prior SARS-CoV-2 infected and seroconverted individuals were included. S1-specific T cell responses and anti-RBD IgG were detectable one year post SARS-CoV-2 infection: 24 spot-forming cells per 10^6 peripheral blood mononuclear cells (SFCs/10^6 PBMCs) after S1 stimulation and anti-RBD IgG concentration of 74 (IQR 36–158) IU/mL. Responses after the first and second vaccination were comparable with S1-specfic T cell responses of 198 (IQR 137–359) and 180 (IQR 103–347) SFCs/10^6 PBMCs, and IgG concentrations of 6792 (IQR 3386–15,180) and 6326 (IQR 2336–13,440) IU/mL, respectively. These responses retained up to four months after vaccination.
Conclusions: Both T cell and IgG responses against SARS-CoV-2 persist for up to one year after COVID-19. A second COVID-19 vaccination in prior-infected individuals did not further increase immune responses in comparison to one vaccination.

Introduction

Immune protection against severe acute respiratory coronavirus-2 (SARS-CoV-2) infection is commonly associated with the presence of neutralising antibodies that bind to the receptor-binding domain (RBD) of the virus' Spike glycoprotein.[1,2] These RBD-bound antibodies prevent interactions between RBD and host's angiotensin-converting enzyme-2 (ACE2), which is a critical process for SARS-CoV-2 cell invasion.[3,4]

In contrast, most coronavirus disease 2019 (COVID-19) immunity studies paid less attention to the role of the cellular component of the adaptive immune system.[5] There is increasing evidence that an effective T cell response is crucial for protection against SARS-CoV-2 infection and severity of disease. For example, the presence of robust SARS-CoV-2-specific T cell responses is associated with successful recovery from COVID-19,[6] whereas lymphopenia, especially of the $CD8^+$ T cell subset, is commonly observed in severe COVID-19 cases.[7–11] In the absence of an effective antiviral T cell response, severe COVID-19 patients present a severe and persistent lung inflammation mediated by highly activated myeloid cells.[12,13] Furthermore, the SARS-CoV-2 Alpha (B.1.1.7 lineage) and Beta (B.1.351 lineage) variants of concern (VOC) partially escaped humoral but not T cell responses in COVID-19 convalescent donors and vaccinees.[14,15] Moreover, the Delta (B.1.617 lineage) variant demonstrated three- to fivefold lower neutralising antibody titres after two BNT162b2 or ChAdOx-1 vaccinations,[16] whereas T cell responses were robust and cross-reactive against the VOC after natural infection or two BNT162b2 vaccinations.[17] Therefore, the assessment of T cell responses might be equally important as the assessment of SARS-CoV-2 specific antibody responses to evaluate one's immune status after natural infection or COVID-19 vaccination.

Most previous SARS-CoV-2 immunity studies assessed SARS-CoV-2-specific immune responses in COVID-19 convalescents up to nine months post-symptom onset (PSO),[18–27] or in healthy individ-

* Corresponding author at: Department of Medical Microbiology and Infection Control, Franciscus Gasthuis and Vlietland, Kleiweg 500, Rotterdam 3045 PM, the Netherlands.
E-mail address: davidsyong@gmail.com (D.S.Y. Ong).

https://doi.org/10.1016/j.jinf.2021.12.003
0163-4453/

uals after administrating COVID-19 vaccinations.[28–31] However, little is known about the persistence of SARS-CoV-2-specific T cell and antibody responses one year after SARS-CoV-2 infection and how COVID-19 vaccinations affect these responses in prior-infected individuals.

This study aimed to describe and compare SARS-CoV-2-specific T cell and antibody responses in a cohort of healthcare workers (HCWs) that suffered from mild to moderate COVID-19 one year ago. Second, we aimed to describe COVID-19 vaccine-induced T cell and antibody responses in our cohort of COVID-19 convalescents.

Methods

Study design

HCWs that suffered from mild to moderate COVID-19 and tested SARS-CoV-2 reverse transcription-quantitative polymerase chain reaction (RT-qPCR) positive approximately one year ago (i.e., between March and July 2020) and in which seroconversion occurred in the following months post diagnosis as described previously were eligible for this study.[32] Ideally, SARS-CoV-2-specific T cell and antibody responses in blood were determined at three time points: before COVID-19 vaccination, two weeks after the first vaccination, and if applicable after the second COVID-19 vaccination.

The study was conducted following the principles of the Declaration of Helsinki, and ethical approval was obtained from the Medical Research Ethical Committee United (protocol number R20.030). All participants provided written informed consent for participation.

PBMC and serum isolation

Whole blood was obtained by venipuncture and was collected in lithium-heparin tubes. Within eight hours after blood collection, serum was isolated from the whole blood sample and peripheral blood mononuclear cells (PBMCs) were isolated using the Ficoll® paque density gradient separation. Cells were washed twice adding pre-heated (37 °C) RPMI 1640 cell culture medium (Gibco) and centrifugation. The pellet was resuspended in pre-heated (37 °C) AIM-V medium (AIM-V® + AlbuMAX® (BSA); Gibco). The PBMC concentration was determined in an automated cell counter (WBC System; HemoCue®), whereafter the PBMCs were diluted in pre-heated (37 °C) AIM-V medium.

SARS-CoV-2 ELISpot

T cell responses against SARS-CoV-2 antigens were assessed by the T-SPOT® Discovery SARS-CoV-2 (Oxford Immunotec). The assay was performed exclusively with materials from the kit, according to the manufacturer's instructions. On day 1, the following stimulators were added in a volume of 50 μL per well: AIM-V as a negative control, phytohemagglutinin as a positive control, and SARS-CoV-2 spike subunit 1 (S1), nucleocapsid protein (N), and membrane protein (M) peptide pools that exclude peptide sequences homologous to endemic coronaviruses. 2.5×10^5 PBMCs in 100 μL AIM-V medium were added to each well. The microtiter plate was incubated for 16–20 h at 37 °C with 5% CO_2 in a humidified atmosphere.

On day 2, cells were washed off the plate with PBS, and the alkaline phosphatase conjugated antibody was added to the wells and was incubated for one hour at 7 °C. The plate was washed with PBS and the substrate was added to the wells and was incubated at room temperature for seven minutes, whereafter the reaction was stopped with demineralised water.

ELISpot spot quantification

Spots were visualised with a digital microscope (Veho® DX1) in a standardised illuminated environment. Images were analysed using FIJI v2.1.0 software[33] and were converted in black-and-white images, whereafter an intensity threshold of 75 and particle size threshold of 5 pixel^2 were set to automatically detect all distinct dark-colored spots using the Particle Analysis tool. The sample was excluded if <100 spots were present in the positive control well. To quantify antigen-specific responses, the number of spots of the negative control well was subtracted from the antigen stimulation wells. The results were expressed as spot-forming cells per 10^6 PBMCs (SFCs /10^6 PBMCs) and were categorised as none (0 SFCs /10^6 PBMCs), weak (1–23 SFCs /10^6 PBMCs), strong (24–99 SFCs /10^6 PBMCs), or very strong ($\geq$100 SFCs /10^6 PBMCs).

SARS-CoV-2 serology

Humoral immune responses were determined with a quantitative ELISA that detected anti-SARS-CoV-2-Spike-RBD IgG antibodies (Beijing Wantai Biological Pharmacy Enterprise). The ELISA was performed according to the manufacturer's instructions in a fully automated microplate processor (EVOLIS; Bio-Rad). The 32 Wantai-units per mL (U/mL) standard, part of the ELISA kit, was serially diluted to prepare six standard concentrations ranging from 32 to 1 U/mL. The standard concentrations were used to create a calibration line, whereafter antibody concentrations in serum samples were assessed. Serum IgG concentrations were converted from U/mL to international units per mL (IU/mL) using the conversion factor of 5.4 as specified by the manufacturer. Serum samples were undiluted, or if necessary, up to 1:1000 diluted to fit in the concentration range of the calibration line. HRP-conjugate was incubated at 37 °C for 30 min and TMB substrate was incubated at 37 °C for 15 min. The reaction was stopped with sulfuric acid and the absorbence was measured at 450 nm.

Statistical analysis

Data were expressed as median with interquartile range (IQR). The Mann-Whitney U test was performed to compare two independent groups. The Kruskal-Wallis test with Dunn's post-hoc analysis was performed to compare two groups within three or more groups. The Wilcoxon signed-rank test was used to compare paired samples. Spearman's rank correlation coefficients (r) were calculated to assess associations between groups and correlation strength was interpreted as negligible (r<0.1), weak (r = 0.11–0.39), moderate (r = 0.40–0.69), strong (r = 0.70–0.89), or very strong (r = 0.90–1.00).[34] All statistic tests were performed at a two-tailed α- level of 0.05 using GraphPad Prism v9 or IBM® SPSS® Statistics v26 for MacOS.

Results

Cohort of prior SARS-CoV-2-infected HCWs

Of 79 HCWs that suffered from mild to moderate COVID-19 one year ago, 20 HCWs did not respond or refused to participate and 4 HCWs had no seroconversion in the first months post-COVID-19 (Fig. 1). The remaining 55 SARS-CoV-2 seroconverted HCWs were included in the study. This cohort had a median age of 43 (IQR 31–55) years at the time of inclusion and consisted of 46 (84%) females.

Some HCWs refused vaccination (n = 9) or already received one (n = 7) or two (n = 16) vaccinations before the study, precluding the assessment of immune responses at all three time points in

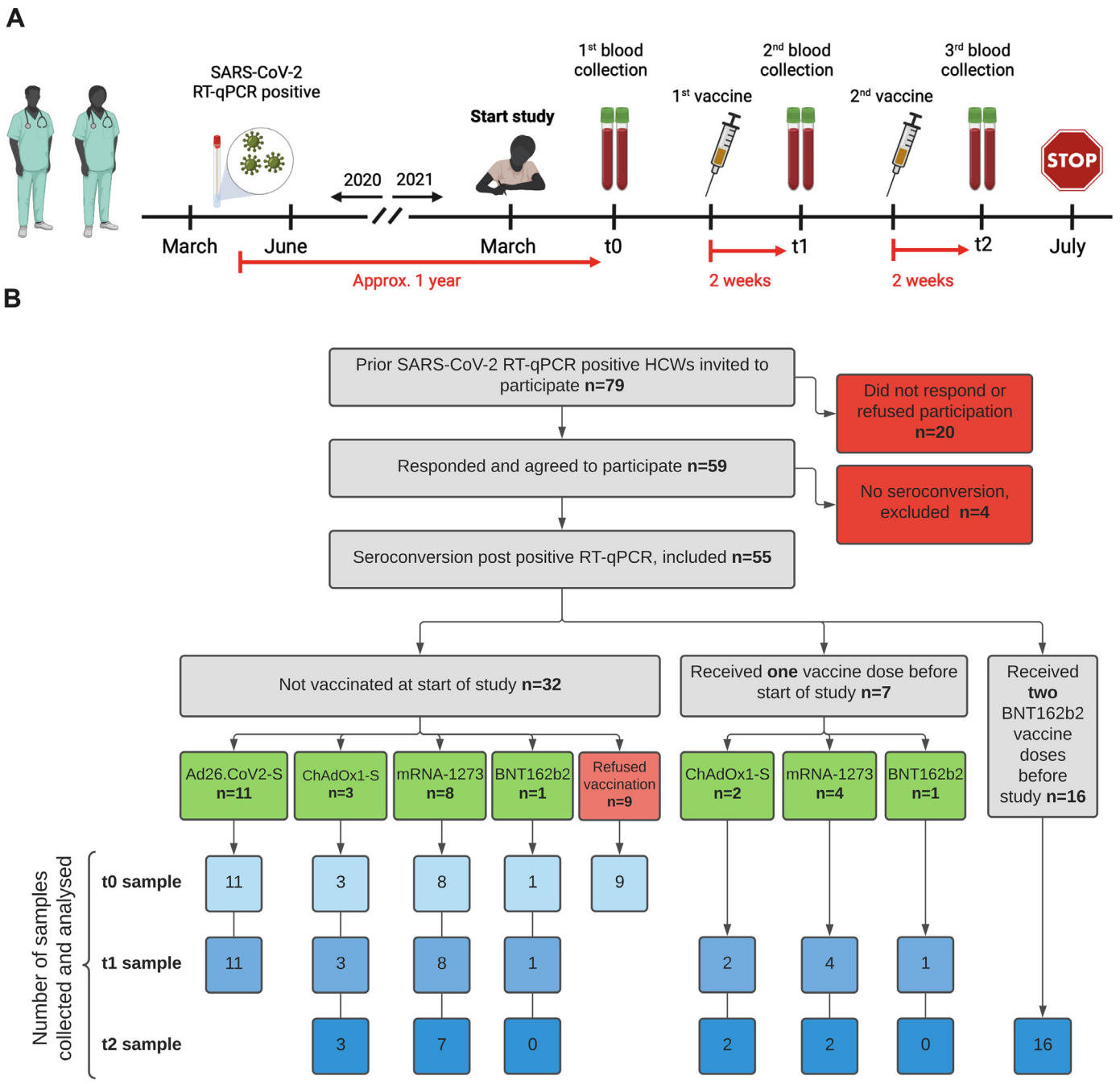

Fig. 1. Study design. (A) Timeline of the study. Blood was collected and immune responses were determined at three time points: before vaccination (t0) and two weeks after the first (t1) and second (t2) vaccination. (B) Flow diagram showing the population and sample size of the study.

these HCWs. Importantly, during the study period national vaccination policy in the Netherlands changed the need for two vaccinations to one vaccination only in prior-infected individuals.[35]

SARS-CoV-2-specific T cell and antibody responses 1 year post COVID-19

We stimulated PBMCs isolated from 32 COVID-19 convalescent vaccination-naïve HCWs. This sub-cohort was tested SARS-CoV-2 RT-qPCR positive 370 (IQR 353–390) days before blood collection. Median SFCs per 10^6 PBMCs were 24 after S1 stimulation and 12 after N or M stimulation (Fig. 2A). All HCWs were at least weak (1–23 SFCs) responsive to S1, of which 53% of HCWs exhibited strong (24–100 SFCs) or very strong (≥100 SFCs) S1-specific responses. In contrast, 30 (93.8%) and 29 (90.6%) HCWs were at least weak responsive to N and M, respectively. In only 9 (28%) and 8 (25%) HCWs strong to very strong responses against N and M were observed, respectively. SARS-CoV-2 T cell responses were present in all HCWs, of which 27 (84%) presented at least weak T cell responses against all three tested antigens (Fig. 2B).

All HCWs presented detectable anti-RBD IgG antibodies one year post SARS-CoV-2 infection (Fig. 2C). The median concentration of anti-RBD IgG in serum was 74 (IQR 36–158) IU/mL. No association between RBD-targeted humoral and S1-targeted cellular responses one year post SARS-CoV-2 infection was detected ($r = 0.0513$, $p = 0.7837$) (Fig. 2D).

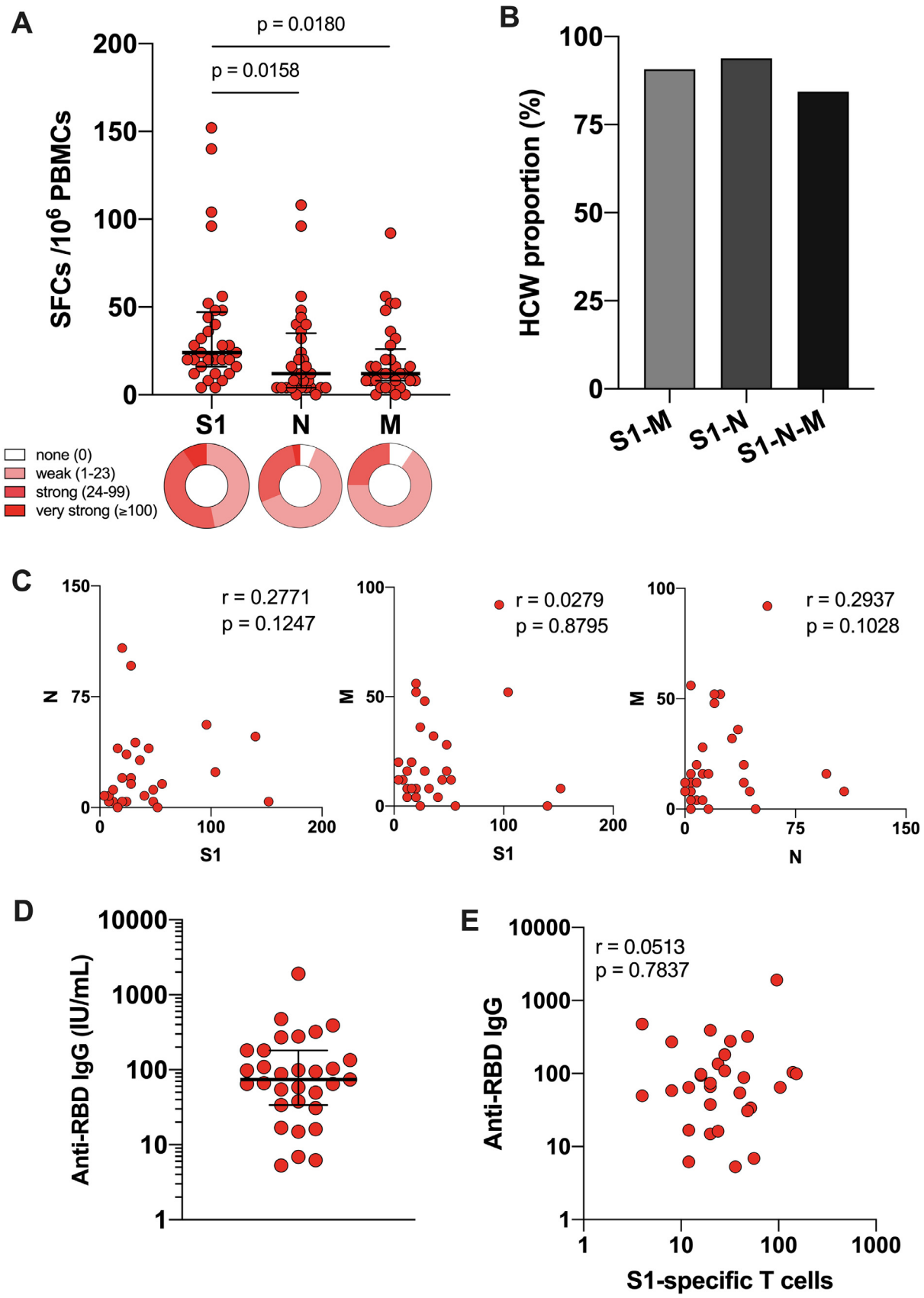

Fig. 2. SARS-CoV-2-specific T cell and antibody responses one year after mild to moderate COVID-19. (A) T cell response magnitude of 32 COVID-19 recovered HCWs to tested SARS-CoV-2 antigens after stimulation for 16–20 h in the ELISpot assay. Each dot represents a COVID-19 convalescent HCW, and doughnut charts represent the proportion of HCWs having none, weak, strong, or very strong responses to tested antigen. Black lines indicate the median with IQR, and data statistical significance was determined using Kruskal-Wallis test with Dunn's post-hoc analysis. (B) Bar chart representing the proportion of HCWs that showed at least weak T cell responses. (C) Associations between antigen-specific responses assessed by Spearman's rank correlation coefficient (*r*). (D) The magnitude of anti-RBD IgG serum concentrations. One serum sample failed in the ELISA, leaving 31 HCWs in the antibody assessment. Black lines indicate median with IQR. (E) Associations between S1-specific T cell and anti-RBD IgG responses assessed by Spearman's rank correlation.

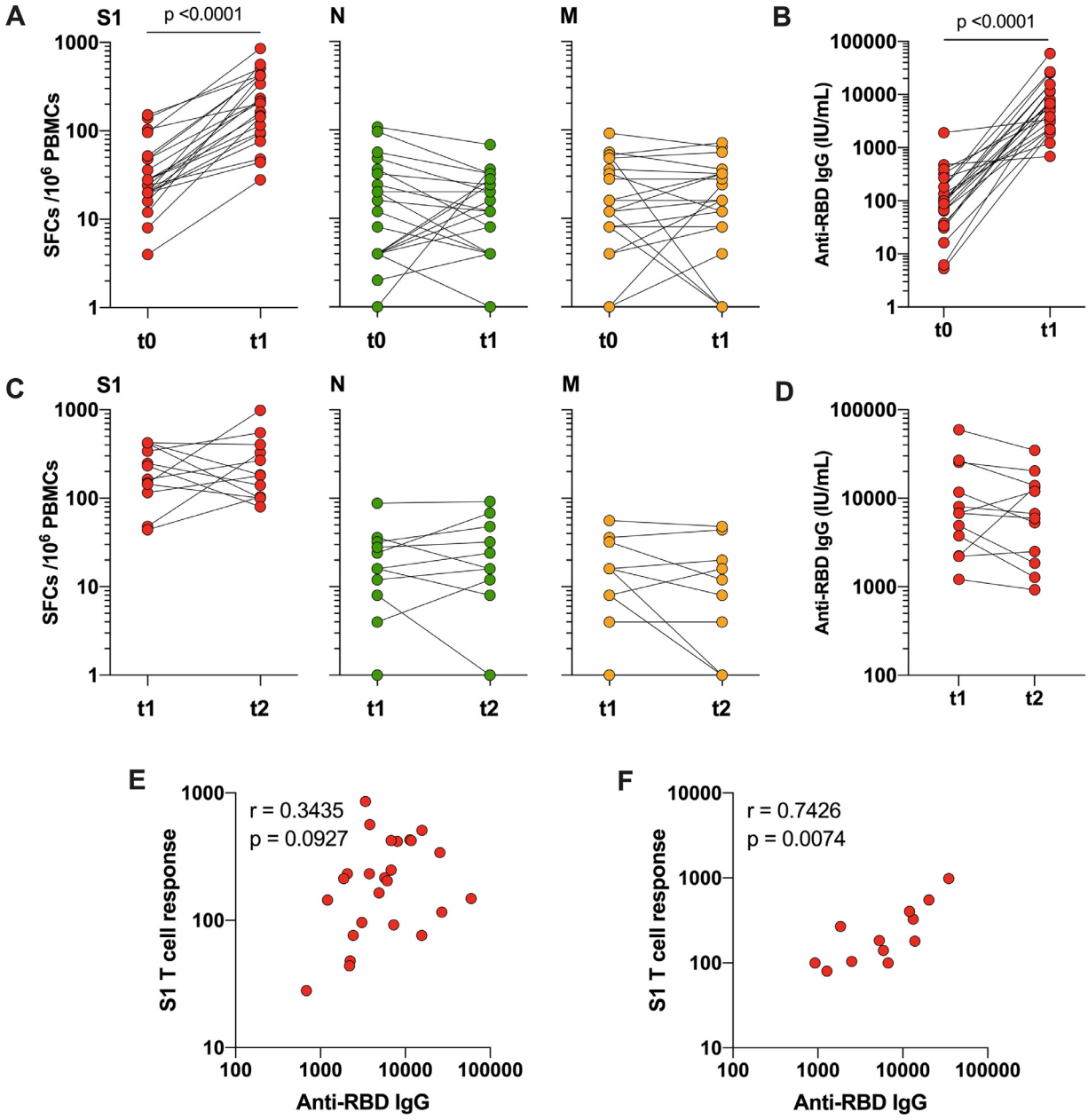

Fig. 3. Vaccine-induced SARS-CoV-2-specific T cell and antibody responses in COVID-19 convalescent HCWs. (A) T cell responses and anti-RBD IgG concentrations (B) median 5 (IQR 2–26) days before (t0) and median 15 (IQR 14–17) days after (t1) one dose of Ad26.COV2-S (n = 11), mRNA-1273 (n = 8), ChAdOx1-S (n = 3), or BNT162b2 (n = 1) vaccine. (C) T cell responses and (D) anti-S1 IgG concentrations against SARS-CoV-2 antigens median 15 (IQR 14–17) days after first vaccination (t1) and median 14 (IQR 14–17) days after second administration (t2) of mRNA-1273 (n = 8) or ChAdOx1-S (n = 4) vaccines. Median number of days between first and second vaccination is 30 (IQR 27–34). Association between S1-specific T cell responses and anti-RBD IgG concentrations at (E) t1 and (F) t2 assessed by Spearman's rank correlation. Statistical significance is determined using Wilcoxon signed-rank test (A-D).

COVID-19 vaccine-induced immune responses in prior SARS-CoV-2-infected and seroconverted individuals

Compared to before vaccination, T cell responses against S1 were significantly higher after the first vaccination with mRNA-1273, BNT162b2, Ad26.CoV2-S or ChAdOx1-S vaccine dose (p<0.0001) (Fig. 3A). Median S1 responses increased from 24 (IQR 20–48) to 204 (IQR 94–420) SFCs /10^6 PBMCs. In contrast, N and M specific T cell responses remained comparable after vaccination. Likewise, anti-RBD IgG concentrations increased significantly after first vaccination (p<0.0001) (Fig 3B). Median anti-RBD IgG concentrations increased from 97 (IQR 44–182) to 5930 (IQR 2442–11,634) IU/mL.

12 HCWs received two mRNA-1273 (n = 8) or ChAdOx1-S (n = 4) vaccine doses during the study. S1-specific T cell responses and anti-RBD IgG concentrations were not significantly different two weeks after the second vaccination, compared to two weeks after the first vaccination (Fig. 3C and D). Median S1-specfic T cell responses changed from 198 (IQR 137–359) to 180 (IQR 103–347) SFCs per 10^6 PBMCs and anti-RBD IgG concentrations changed from 6792 (IQR 3386–15,180) to 6326 (IQR 2336–13,440) IU/mL.

Furthermore, we observed a non-significant weak association between S1-specific T cell and anti-RBD IgG responses after the first vaccination (r = 0.3435, p = 0.0927) and a statistically significant strong association after the second vaccination (r = 0.7426, p = 0.0074) (Fig. 3E and F).

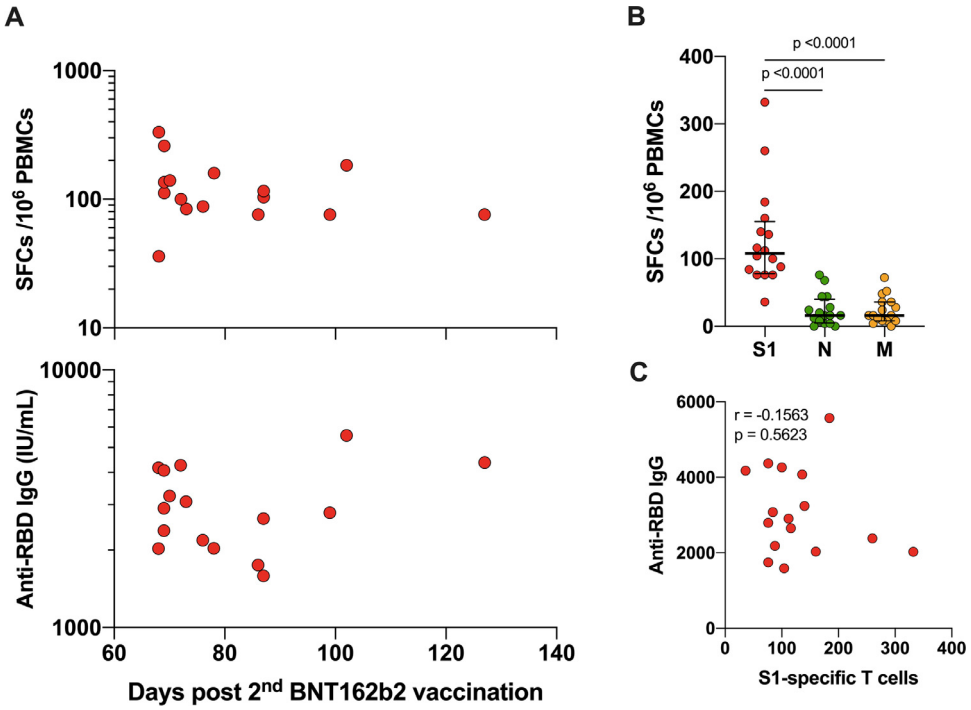

Fig. 4. Persistence of vaccine-induced SARS-CoV-2-specific T cell responses in COVID-19 convalescent HCWs. (A) S1-specific T cell and anti-RBD IgG responses of COVID-19 convalescent HCWs (n = 16) who received two BNT162b2 vaccinations 68–127 days before blood collection. (B) Comparison of SARS-CoV-2 antigen-specific IFNγ responses. Black lines indicate median with IQR, and statistical significance was determined using Mann-Whitney U test. (C) Association between T cell and anti-RBD IgG responses was assessed by Spearman's rank correlation.

Due to small numbers of measurements at similar timing after the second vaccination, we could not make an adequate comparison between all various vaccine types. Nevertheless, we observed significantly higher anti-RBD IgG concentrations, 10,001 (IQR 5774–15,525) and 1276 (IQR 1224–2498) IU/mL (p = 0.0295), two weeks after the second dose of the mRNA-1273 and ChAdOx1-S vaccine, respectively.

Durability of COVID-19 vaccine-induced immune responses in COVID-19 convalescents

16 HCWs tested SARS-CoV-2 RT-qPCR positive at a median of 366 (IQR 357–380) days before blood collection and were already fully vaccinated at a median of 75 (IQR 69–87, range 68–127) days before blood collection. These HCWs received a second dose of Pfizer's BNT162b2 vaccine at a median of 27 (IQR 25–28) days after the first dose and showed overall maintained T cell and antibody responses during the four months post second vaccination (Fig. 4A). Moreover, S1 induced significantly higher T cell reactivity compared to N and M; median SFCs of 108 for S1 versus 16 for both N and M (Fig. 4B). No significant correlation was found between S1-specific T cell responses and anti-RBD IgG concentrations (r=−0.1563, p = 0.5623) (Fig. 4C).

Discussion

Here, we demonstrated that all mild to moderate COVID-19 convalescents presented SARS-CoV-2-specific T cell and IgG responses one year after disease, which were adequately boosted by a single mRNA or viral vector COVID-19 vaccination. Furthermore, we observed retained T cell and antibody responses up to four months after the second BNT162b2 vaccination in prior-infected individuals.

Whereas previous studies mainly focused on the persistence of T cell and antibody responses after SARS-CoV-2 infection weeks to months PSO,[18–27] this study is one of the first to assess both SARS-CoV-2-specific T cell and antibody responses one-year post-SARS-CoV-2 infection. In addition, this study assessed vaccination-induced immune responses in prior-infected individuals.

In line with our findings, previous studies also reported retained SARS-CoV-2-specific T cell and antibody responses in prior-infected individuals up to nine months PSO.[36–38] Interestingly, we found that S1-specific T cell responses were not associated with anti-RBD IgG responses, indicating heterogeneity in the durability of humoral and cellular SARS-CoV-2 immunity. Moreover, a previous study showed discordancy between S1 IgG and T cell responses in prior SARS-CoV-2-infected individuals, in which S1 IgG levels increased with age in females while T cell responses increased with age in males.[22] Furthermore, our cohort exhibited heterogeneous T cell responses, which could be related to sex and age,[39] or may be caused by unrecognised re-exposure to SARS-CoV-2 since the virus was highly prevalent in the Netherlands from September 2020 to May 2021.[40]

Irrespective of the COVID-19 vaccine type, anti-RBD IgG and S1-specific T cell responses became considerably stronger two weeks after the first vaccination, whereas these responses did not further increase after the second COVID-19 vaccination in prior-infected HCWs. Previous studies on COVID-19 mRNA vaccines showed comparable findings of prior-infected individuals already exhibiting peak immune responses after the first vaccination and responses not further increasing after a second vaccination, indicating the redundancy of a second COVID-19 vaccination in prior-infected individuals.[41,42]

Even though our results demonstrated durable immune responses after infection and vaccination, it remains questionable whether sufficient protection against all SARS-CoV-2 variants is achieved. For example, SARS-CoV-2 Delta variant infections also emerged in partially and fully vaccinated individuals.[43–46] Neutralising antibodies of COVID-19 convalescent individuals showed to be fourfold less potent against the Delta variant than the Alpha

variant one year PSO, and there was barely an inhibitory effect against the Delta variant after a single BNT162b2 or ChAdOx1-S vaccination.[16,47]

There are some limitations to consider in our study. First, we did not assess neutralising antibody responses in our HCWs cohort. However, in our earlier work, the Wantai ELISA showed high agreement with a surrogate neutralising antibody test.[32] Second, not all HCWs received the first and second vaccination in our study, and only few HCWs received BNT162b2 and ChAdOx1-S vaccines. We were dependant on national COVID-19 vaccination policies and could not split our cohort into even groups per vaccine type. Also, the national policy changed the need for two vaccinations to one vaccination only in prior-infected individuals during the study period, impeding the assessment of immune responses two weeks after second vaccination in a proportion of HCWs. Nevertheless, our findings suggest the redundancy of the second vaccination in prior SARS-CoV-2 infected individuals and may guide COVID-19 vaccination policies.

In conclusion, T cell responses and anti-RBD IgG against SARS-CoV-2 persist for up to one year after COVID-19. Also, irrespective of the vaccine type, a single COVID-19 vaccination induced robust S1-specific T cell responses and anti-RBD IgG antibodies in COVID-19 convalescents, whereas responses did not further increase after the second vaccination. Future research must longitudinally assess SARS-CoV-2-specific immune responses in prior-infected individuals and elucidate how long a single COVID-19 vaccination could be considered equally effective as full vaccination.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

CRediT authorship contribution statement

Willem A. Mak: Data curation, Visualization, Formal analysis, Writing – original draft, Writing – review & editing. **Johannes G.M. Koeleman:** Conceptualization, Visualization, Supervision, Writing – review & editing. **Marijke van der Vliet:** Data curation, Writing – review & editing. **Frans Keuren:** Formal analysis, Writing – review & editing. **David S.Y. Ong:** Conceptualization, Visualization, Supervision, Writing – original draft, Writing – review & editing.

Funding

Franciscus Gasthuis & Vlietland, Rotterdam, the Netherlands.

Acknowledgments

We would like to thank Marie-Jozé Rentmeester for her assistance in the logistic process during the study.

References

1. Ong David SY, Fragkou Paraskevi C, Schweitzer Valentijn A, Chemaly Roy F, Moschopoulos Charalampos D, Chrysanthi S. How to interpret and use COVID-19 serology and immunology tests. *Clin Microbiol Infect* 2021;**27**(7):981–6. doi:10.1016/j.cmi.2021.05.001.
2. Khoury David S, Deborah C, Arnold R, Schlub Timothy E, Wheatley Adam K, Juno Jennifer A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* 2021;**27**:1205–11. doi:10.1038/s41591-021-01377-8.
3. Markus H, Hannah KW, Simon S, Nadine K, Tanja H, Sandra E, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 2020;**181**(2):271–80 e8. doi:10.1016/j.cell.2020.02.052.
4. Filippo S, Aurora D, Felice A, Lucio P, Gabriella MM, Mario C, et al. ACE2: the major cell entry receptor for SARS-CoV-2. *Lung* 2020;**198**(6):867–77. doi:10.1007/s00408-020-00408-4.
5. Takuya S, André PP, Olga RB, Kristoffer S, Jean-Baptiste G, Annika O, et al. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. *Cell* 2020;**183**:158–68. doi:10.1016/j.cell.2020.08.017.
6. Alba G, Daniela W, Ramirez S, Jose M, Dan J, Rydyznski MC, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell* 2020;**181**(7):1489–501. doi:10.1016/j.cell.2020.05.015.
7. Shaohua L, Lina J, Xi L, Fang L, Yijin W, Boan L, et al. Clinical and pathological investigation of patients with severe COVID-19. *JCI Insight* 2020;**5**(12):1–13. doi:10.1172/jci.insight.138070.
8. Altmann Daniel M, Boyton Rosemary J. SARS-CoV-2 T cell immunity: specificity, function, durability, and role in protection. *Sci Immunol* 2020;**5**(49):2–7. doi:10.1126/sciimmunol.abd6160.
9. Daniela W, Schmitz K, Raadsen M, Alba G, Okba N, Henrik E, et al. Phenotype and kinetics of SARS-CoV-2-specific T cells in COVID-19 patients with acute respiratory distress syndrome. *Sci Immunol* 2020;**5**(48):1–14. doi:10.1126/SCIIMMUNOL.ABD2071.
10. Oja Anna E, Anno S, Ghandour Cherien A, Kragten Natasja AM, Hogema Boris M, Nossent Esther J, et al. Divergent SARS-CoV-2-specific T- and B-cell responses in severe but not mild COVID-19 patients. *Eur J Immunol* 2020;**50**(12):1998–2012. doi:10.1002/eji.202048908.
11. Urra JM, Cabrera CM, Porras L, Ródenas I. Selective CD8 cell reduction by SARS-CoV-2 is associated with a worse prognosis and systemic inflammation in COVID-19 patients. *Clin Immunol* 2020;**217**:1–5. doi:10.1016/j.clim.2020.108486.
12. Antonio B, Nina L, Martin Q, Tan A. SARS-CoV-2-specific T cells in infection and vaccination. *Cell Mol Immunol* 2021:1–6. doi:10.1038/s41423-021-00743-3.
13. Alessandro S, Shane C. Adaptive immunity to SARS-CoV-2 and COVID-19. *Cell* 2021;**184**(4):861–80. doi:10.1016/j.cell.2021.01.007.
14. Geers D, Sci I, Daryl G, Shamier MC, Susanne B, Den HG, et al. SARS-CoV-2 variants of concern partially escape humoral but not T-cell responses in COVID-19 convalescent donors and vaccinees. *Sci Immunol* 2021;**6**(59):1–22. doi:10.1126/sciimmunol.abj1750.
15. Alison T, John S, Nils M, Dawen Y, Yun Z, Dan Jennifer M, et al. Impact of SARS-CoV-2 variants on the total CD4+ and CD8+ T cell reactivity in infected or vaccinated individuals. *Cell Rep Med* 2021;**2**:1–12. doi:10.1016/j.xcrm.2021.100355.
16. Delphine P, David V, Artem B, Isabelle S, Florence GB, Michael RM. Reduced sensitivity of SARS-CoV-2 variant delta to antibody neutralization. *Nature* 2021;**596**:276–80. doi:10.1038/s41586-021-03777-9.
17. Jordan Stanley C, Bong-Ha S, Gadsden Terry-Ann M, Maggie C, Anna P, Le Catherine N, et al. T cell immune responses to SARS-CoV-2 and variants of concern (Alpha and Delta) in infected and vaccinated individuals. *Cell Mol Immunol* 2021:1–3. doi:10.1038/s41423-021-00767-9.
18. Yun T, Feng L, Xiaoguang Xu, Yun L, Weijin H, Zhaoqin Z, et al. Durability of neutralizing antibodies and T-cell response post SARS-CoV-2 infection. *Front Med* 2020;**14**(6):746–51. doi:10.1007/s11684-020-0822-5.
19. Tatjana B, Annika N, Heitmann Jonas S, Yacine M, Malte R, Jens B, et al. T cell and antibody kinetics delineate SARS-CoV-2 peptides mediating long-term immune responses in COVID-19 convalescent individuals. *Sci Transl Med* 2021;**13**:1–12. doi:10.1126/scitranslmed.abf7517.
20. Jianmin Z, Alexander D, Hayden P, Verma K, Long H, Jusnara B, et al. Robust SARS-CoV-2-specific T cell immunity is maintained at 6 months following primary infection. *Nat Immunol* 2021;**22**:620–6. doi:10.1038/s41590-021-00902-8.
21. Lin JX, Lin WG, Na ZX, Hu YF, Lin Y, Qiang KZ, et al. Lasting antibody and T cell responses to SARS-CoV-2 in COVID-19 patients three months after infection. *Nat Commun* 2021;**12**(1):1–10. doi:10.1038/s41467-021-21155-x.
22. Catherine R, Sci I, Catherine RJ, Leo S, Joseph G, Corinna P, et al. Discordant neutralizing antibody and T cell responses in asymptomatic and mild SARS-CoV-2 infection. *Sci Immunol* 2020;**5**(54):1–18.
23. Natalia S, Antonio P, Likun Du, Hui W, Makiko KB, Juni A, et al. Persistence of SARS-CoV-2-specific B and T cell responses in convalescent COVID-19 patients 6–8 months after the infection. *Med* 2021;**2**(3):281–95. doi:10.1016/j.medj.2021.02.001.
24. Baweleta I, Kento A, Michelle Z, Alainna J, Bhavisha R, Wang Jenny H, et al. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. *Sci Immunol* 2020;**5**(52):1–20. doi:10.1126/sciimmunol.abe5511.
25. Casado L, Pilar V, Hector V, Johannes H, Amaranta M, Marina FE, et al. Progressive and parallel decline of humoral and T-cell immunity in convalescent healthcare workers with asymptomatic or mild-to-moderate severe acute respiratory syndrome coronavirus 2 infection. *J Infect Dis* 2021;**224**:241–5. doi:10.1093/infdis/jiab242.
26. Gaëlle B, Pilar M, Thomas H, Oliveira Thiago Y, Dennis SB, Christian G, et al. Persistent cellular immunity to SARS-CoV-2 infection. *J Exp Med* 2021;**218**(4):1–11. doi:10.1084/JEM.20202515.
27. Dan J, Jose M, Yu K, Hastie K, Dawen Y, Faliti Caterina E, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science* 2021;**371**(6529):1–22 (80). doi:10.1126/science.abf4063.
28. Ugur S, Alexander M, Evelyna D, Isabel V, Kranz L, Mathias V, et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. *Nature* 2020;**586**(7830):594–9. doi:10.1038/s41586-020-2814-7.
29. Stephenson K, Le Gars Mathieu SJ, De Groot Anne Marit HD, Carla T, et al. Immunogenicity of the Ad26.COV2.S Vaccine for COVID-19. *JAMA* 2021. doi:10.1001/jama.2021.3645.
30. Anderson E, Rouphael N, Widge A, Jackson L, Roberts P, Mamodikoe M, et al. Safety and immunogenicity of SARS-CoV-2 mRNA-1273 vaccine in older adults. *N Engl J Med* 2020;**383**(25):2427–38. doi:10.1056/nejmoa2028436.
31. Ramasamy Maheshi N, Minassian Angela M, Ewer Katie J, Flaxman Amy L, Folegatti Pedro M, Owens Daniel R, et al. Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old

adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial. *Lancet* 2020;**396**(10267):1979–93. doi:10.1016/S0140-6736(20)32466-1.
32. Ong David SY, Frans K, van der Vliet Marijke M, Koeleman Johannes GM. SARS-CoV-2 antibody response dynamics and heterogeneous diagnostic performance of four serological tests and a neutralization test in symptomatic healthcare workers with non-severe COVID-19. *J Clin Virol* 2021;**141**:1–3. doi:10.1016/j.jcv.2021.104904.
33. Johannes S, Ignacio AC, Erwin F, Verena K, Mark L, Tobias P, et al. Fiji: an open-source platform for biological-image analysis. *Nat Methods* 2012;**9**(7):676–82. doi:10.1038/nmeth.2019.
34. Patrick S, Boer C, Lothar A. Correlation coefficients: appropriate use and interpretation. *Anesth Analg* 2018;**126**(5):1763–8. doi:10.1213/ANE.0000000000002864.
35. National Institute for Public Health and the Environment (RIVM), COVID-19 vaccination regulations. [cited 02/10/2021]; Available at: https://www.rivm.nl/en/covid-19-vaccination/vaccines/efficacy-and-protection
36. Cohen K, Linderman S, Zoe M, Julie C, Lilin L, Grace M, et al. Longitudinal analysis shows durable and broad immune memory after SARS-CoV-2 infection with persisting antibody responses and memory B and T cells. *Cell Rep Med* 2021;**2**(7):100354. doi:10.1016/j.xcrm.2021.100354.
37. Tyler SJ, Renata V, Wanda C, Puran C, Muvva Jagadeeswara R, Maleki Kimia T, et al. SARS-CoV-2-specific humoral and cellular immunity persists through 9 months irrespective of COVID-19 severity at hospitalisation. *Clin Transl Immunol* 2021;**10**(7):1–18. doi:10.1002/cti2.1306.
38. Lin Y, Guo-Lin W, Yuan S, Zhuang-Ye W, Bing-Dong Z, Li-Jun D, et al. Persistence of antibody and cellular immune responses in coronavirus disease 2019 patients over nine months after infection. *J Infect Dis* 2021;**224**(4):586–94. doi:10.1093/infdis/jiab255.
39. Takehiro T, Ellingson M, Patrick W, Benjamin I, Carolina L, Jon K, et al. Sex differences in immune responses that underlie COVID-19 disease outcomes. *Nature* 2020;**588**(7837):315–20. doi:10.1038/s41586-020-2700-3.
40. National Institute for Public Health and the Environment (RIVM), weekly SARS-CoV-2 figures. [cited 02/10/2021]; Available at: https://www.rivm.nl/en/coronavirus-covid-19/weekly-figures
41. Goel R, Apostolidis S, Painter M, Divij M, Ajinkya P, Oliva K. Distinct antibody and memory B cell responses in SARSCoV-2 naïve and recovered individuals following mRNA vaccination. *Sci Immunol* 2021;**6**(58):1–19. doi:10.1126/sciimmunol.abi6950.
42. Maria P, Candice C, Jonathan B, Alison C, Sarah G. Effect of previous SARS-CoV-2 infection on humoral and T-cell responses to single-dose BNT162b2 vaccine. *Lancet* 2021;**397**(10280):1178–81. doi:10.1016/S0140-6736(21)00502-X.
43. Iivo H, Sohvi K, Pirjo A, Janne M, Carita S, Niina I, et al. An outbreak caused by the SARS-CoV-2 Delta variant (B. 1. 617. 2) in a secondary care hospital in Finland. *Eurosurveillance* 2021;**26**(30):1–6. doi:10.2807/1560-7917.ES.2021.26.30.2100636.
44. Williams S, Amoolya V, Ladhani S, De O, Nalini I, Felicity A, et al. An outbreak caused by the SARS-CoV-2 Delta (B. 1.617. 2) variant in a care home after partial vaccination with a single dose of the COVID-19 vaccine Vaxzevria, London, England, April 2021. *Eurosurveillance* 2021;**26**(27):1–5. doi:10.2807/1560-7917.ES.2021.26.27.2100626.
45. Kendra D, Mike M, Ozair N, Dakota M, Jolianne S. SARS-CoV-2 B1. 617. 2 (Delta) variant COVID-19 outbreak associated with a gymnastics facility — Oklahoma, April – May 2021. *Morb Mortal Wkly Rep* 2021;**70**(28):1004–7.
46. Samuel A, Stéphanie H, Vincent F, Laura V, Sabine T, Bénédicte R, et al. Rapid spread of the SARS-CoV-2 Delta variant in some. *Eurosurveillance* 2021;**26**(28):1–5. doi:10.2807/1560-7917.ES.2021.26.28.2100573.
47. Lopez BJ, Nick A, Charlotte G, Eileen G, Ruth S, Simon T, et al. Effectiveness of COVID-19 vaccines against the B.1.617.2 (Delta) variant. *N Engl J Med* 2021;**385**(7):585–94. doi:10.1056/NEJMoa2108891.