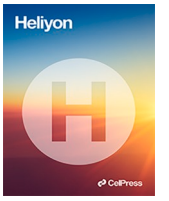
Heliyon 10 (2024) e29116

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Review article

Study of the cellular and humoral immune responses to SARS-CoV-2 vaccination

Faustine Montmaneix-Engels [a,b,*], Chloé Dimeglio [a,c], Laeticia Staes [c], Isabelle Da Silva [c], Marion Porcheron [c], Isabelle Jougla [d], Fabrice Hérin [d,e], Jacques Izopet [a,b,c]

[a] *INSERM UMR1291-CNRS UMR5051-University Toulouse III, Toulouse Institute for Infectious and Inflammatory Diseases, 31300, Toulouse, France*
[b] *Toulouse III Paul Sabatier University, 31062, Toulouse, France*
[c] *CHU Toulouse, Purpan Hospital, Virology Laboratory, 31300, Toulouse, France*
[d] *Occupational Diseases Department, Toulouse University Hospital, 31000, Toulouse, France*
[e] *UMR1295, Joint Research Unit INSERM- University Toulouse III Paul Sabatier, Centre for Epidemiology and Research in Population Health Unit (CERPOP), 31000, Toulouse, France*

ARTICLE INFO

Keywords:
COVID-19 vaccination
SARS-CoV-2
Cellular immunity
Humoral immunity
Interferon gamma release assay

ABSTRACT

Our understanding of cellular immunity in response to COVID-19 infection or vaccination is limited because of less commonly used techniques. We investigated both the cellular and humoral immune responses before and after the administration of a third dose of the SARS-CoV-2 vaccine among a group of healthcare workers. Cellular immunity was evaluated using the VIDAS interferon-gamma (IFNγ) RUO test, which enables automated measurement of IFNγ levels after stimulating peripheral blood lymphocytes.

Booster doses significantly enhanced both cellular and humoral immunity. Concerning cellular response, the booster dose increased the percentage of positive IFNγ release assay (IGRA) results but no difference in IFNγ release was found. The cellular response was not associated with protection against SARS-CoV-2 infection. Interestingly, vaccinated and infected healthcare workers exhibited the highest levels of anti-spike and neutralizing antibodies.

In conclusion, the IGRA is a simple method for measuring cellular immune responses after vaccination. However, its usefulness as a complement to the study of humoral responses is yet to be demonstrated in future research.

1. Introduction

The COVID-19 pandemic caused by the SARS-CoV-2 virus had a profound impact on global health, with millions of cases and deaths reported worldwide. Vaccination against COVID-19 is a crucial strategy for controlling the spread of the virus and reducing its burden. Currently available vaccines are highly effective in preventing severe disease, hospitalization, and death.

Vaccination is based on the induction of humoral and cellular immune responses involving B and T cells. B cells participate in the development of the humoral immune response, which involves the production of antibodies specifically directed against the infectious

* Corresponding author.Toulouse III Paul Sabatier University, 31062, Toulouse, France.
E-mail address: fmontmaneix@etud.univ-angers.fr (F. Montmaneix-Engels).

https://doi.org/10.1016/j.heliyon.2024.e29116
Received 11 January 2024; Received in revised form 29 March 2024; Accepted 1 April 2024
Available online 2 April 2024

agent in response to viral infection or vaccination. Among these antibodies, we distinguished neutralizing antibodies (NAbs) that bind to the surface of the virus and block its entry into the target cell. The anti-SARS-CoV-2 antibody titer was measured to assess the effectiveness of the humoral immunity acquired after vaccination and/or infection [1]. The cellular immune response is mediated by cytotoxic $CD8^+$ and $CD4^+$ T cells, which cooperate with other immune cells. Interferon gamma produced by these cells is an essential cytokine in the immune response. Its measurement after stimulation with specific antigens enables the measurement of cellular immune responses [2,3].

In France, two mRNA vaccines Moderna mRNA-1273 [4] and Pfizer/BioNTech BNT162b2 [5,6], are widely used. Several studies have demonstrated that spike-specific antibody and NAb responses are detected 7–14 days after vaccination and peak approximately 4 weeks later [7,8]. Antibodies, more specifically NAbs, are correlated with protection against SARS-CoV-2 infection and COVID-19 in the first month post-vaccination [9,10]. However, studies have shown that the neutralizing titer and receptor-binding domain (RBD) immunoglobulin G (IgG) declined 6 months after vaccination [11,12], in addition to the emergence of variants of concern (VOCs) which escape to antibodies induced by vaccination [11,13]. For those reasons, a 3-dose mRNA vaccine regimen is recommended. COVID-19 vaccines can also induce strong T cell responses [14]. After two doses of mRNA COVID-19 vaccines, spike specific $CD4^+$ and $CD8^+$ T cell responses were detected in almost 100% and 70–90% of cases, respectively, and were detectable 6 months later [14–17].

Unlike humoral responses, studies have shown that the cellular response to SARS-CoV-2 variants is preserved in vaccinated patients. Indeed, SARS-CoV-2-specific T cells recognize preserved spike protein epitopes that are found not only among variants of interest, but also among common cold coronaviruses [18,19]. The capacity of T cells to recognize Omicron [16,20,21] and all other VOCs [22,23] may explain the high level of protection against severe illness and hospitalization [24,25] observed during the Omicron wave of the COVID-19 pandemic. Although cellular immunity appears to play an important role in vaccine immunity, its assessment focuses on humoral immunity. It is also necessary to develop similar techniques to evaluate anti-SARS-CoV-2 cellular immunity. Currently, the SARS-CoV-2 specific cellular immune response is mainly evaluated after lymphocyte stimulation through the measurement of IFNγ levels by ELISPOT [26], intracellular cytokine staining (ICS) [26], and activation-induced markers (AIMs) [26], which are complex techniques not suitable for routine use. In contrast, the IFNγ release assay is already routinely used to evaluate the cell-mediated immune response to Mycobacterium tuberculosis [27] or cytomegalovirus [2]. Various studies indicate that the IGRA test is a sensitive and specific method for detecting cell-mediated immune response by measuring the release of IFNγ after stimulation of peripheral blood-specific T cells using SARS-CoV-2 peptides [3,28,29].

In this prospective study, we analyzed humoral and cellular responses on the day of and one month after a third dose of mRNA vaccine in healthcare workers (HCWs). Cellular responses in whole blood samples were assessed using the IGRA. The results of this study may help better understand the efficacy of the third dose of the vaccine in preventing COVID-19.

2. Methods

2.1. Participants and sample collection

We analyzed the antibody responses of 40 vaccinated HCWs with no history of SARS-CoV-2 infection, as identified by negative antinucleocapsid serology. Blood samples were collected on the day of the third vaccine dose (20 January 2021–18 February 2022) and one month later (18 February 2022–15 March 2022). For individuals under 30 years old, the booster dose consisted of 30 μg of BNT162b2, Comirnaty® (Pfizer/BioNTech), while health care workers aged 30 years and older received 100 μg of mRNA-1273 (Moderna). Cellular responses were analyzed before the third dose for 24/40 (60%) HCWs and for all of HCWs one month later. Questionnaires regarding the vaccination, infection, and drug status of individuals were collected at both sampling times (Supplementary Data). The reporting of SARS-CoV-2 infection was confirmed by nasopharyngeal swab samples collected at Toulouse University Hospital from individuals with symptoms or in contact with an infected person, which was tested using a PCR assay. Biological materials and clinical data were obtained only for standard virological monitoring. The data were analyzed using an anonymized database. This protocol does not require written informed consent according to French Public Health Law (CSP Art L 1121-1.1).

2.2. Whole blood IGRA

Whole blood samples were collected in heparinized lithium tubes. Whole blood (300 μL) was stimulated using stimulation reagents (300 μL) (VIDAS COVID STIMULATION RUO) within 8 h after blood collection. Peptides from the wild-type SARS-CoV-2 spike protein (genome reference sequence: NC_045512.2) were used to stimulate SARS-CoV-2 specific lymphocytes. Whole blood samples were stimulated with mitogen as a positive control and IGRA solution as a negative control. After a 22–24 h incubation at 35/39 °C under 5% of CO_2, the separated plasma was used to determine IFNγ concentration by enzyme linked fluorescent assay (ELFA) using the VIDAS automated platform (VIDAS IFNγ RUO, bioMérieux). The analytical measurement interval of the VIDAS IFNγ RUO assay was 0.10–8.00 IU/mL with a detection limit of 0.08 IU/mL. Whole blood IGRA positivity thresholds were defined as 0.13 IU/mL according to the manufacturer's recommendations. The response was defined as positive when the IFNγ concentration of the test was above or equal to the threshold and the negative control was below 0.08 IU/mL [3,30].

2.3. Neutralization assay

Neutralizing antibody titers were assessed by endpoint dilution using Vero cells (ATCC CCL-81™) and a clinical Omicron BA.1 strain (EPI_ISL_10316329) [31–33]. Briefly, 10^4 Vero cells in 96-well plates were mixed with a virus suspension (100 $TCID_{50}$) and

2-fold serial dilutions (1:2 to 1:1024) of the test serum and incubated for 6 d at 37 °C. The plates were examined to identify wells showing cytopathic effects (CPEs) [34]. NAb titer was defined as the reciprocal of the highest serum dilution that protected cells from CPEs.

2.4. Antibodies against SARS-CoV-2 spike and nucleocapsid protein

Anti-spike IgG concentrations were measured using a chemiluminescent microparticle immunoassay (IgG II Quant, Alinity, Abbott, Sligo, Ireland). The concentrations expressed as AU/mL were converted to BAU/mL using the conversion factor (0.142), as recommended by the manufacturer [32,33]. Anti-nucleocapsid antibodies were detected using the SARS-CoV-2 IgG assay (Alinity, Abbott, Sligo, Ireland). An index (S/C) of $\geq$1.4 was considered positive.

2.5. Statistical analysis

We used GraphPad Prism 7 software (version 7.03 for Windows, GraphPad Software, Inc.) and RStudio (version April 1, 1717) for statistical analyses. Mann-Whitney U-tests were used to compare two independent groups. The Wilcoxon signed-rank test was used to compare two paired groups. McNemar's test with continuity correction was used to determine whether the paired proportions were different. Values are expressed as medians and interquartile ranges [Q1-Q3] for quantitative variables and N (%) for qualitative variables. Correlations were identified using the Spearman's test. Regarding the measurement of IFNγ, the statistical tests were performed on the concentrations within analytical measurement interval. *P*-values of <0.05 were considered statistically significant.

3. Results

3.1. Population characteristics

Among the 40 HCWs included, the median age was 38.5 [IQR: 33–43.7] and 31 (77.5%) were women. Six HCWs (15%) had been infected with SARS-CoV-2 during the study (identified by positive PCR or the presence of antinucleocapsid antibodies 4 weeks after the 3rd dose). The breakthrough infection occurs 7 days [IQR: 3.5–12] after the third dose. Most of them (5/6, 83.3%) received a mRNA-1273 third dose (i.e., a booster) (Table 1). Similarly, most of the 34 vaccinated uninfected HCWs (28/34, 82.3%) received a mRNA-1273 third dose. Third doses of COVID-19 vaccine were given 160.5 days [IQR: 147.5–184.8] after the last dose of vaccine. The humoral immune response was assessed in every HCWs on the day of the third dose and one month later (28 days [IQR: 28–34]). Cellular immune response was assessed in 3/6 (50%) of the vaccinated infected HCWs and 21/34 (61.8%) of the vaccinated uninfected HCWs the day of the third dose, and in every HCW one month later.

3.2. Cellular response

Before the third dose, a concentration of IFNγ above the limit of quantification (0.1 IU/mL) was observed for 42.9% (9/21) of vaccinated uninfected HCWs and for 100% (3/3) of vaccinated infected HCWs (Fig. 1). After the third dose, 85.3% (29/34) of vaccinated uninfected HCWs and 100% (6/6) of vaccinated infected HCWs had a concentration of IFNγ above the limit of quantification. No difference was found between IFNγ release before and after the third dose in vaccinated uninfected HCWs (0.29 [IQR: 0.13–0.46] vs. 0.42 [IQR: 0.19–1.38], $p > 0.05$, N = 9) and in vaccinated infected HCWs (0.28 [IQR: 0.16–0.37] vs. 1.37 [IQR: 0.20–3.49], $p > 0.05$, N = 3) (Fig. 1). The proportion of positive IGRA increased with the third dose in vaccinated uninfected HCWs (33.3% vs. 76.2%, $p < 0.001$, N = 21) (Fig. 1). All vaccinated infected HCWs showed positive IGRA results before and after the third dose (Fig. 1). No difference in concentration of IFNγ was found between vaccinated uninfected and infected HCWs after the third dose (0.31 [IQR: 0.19–0.69], N = 29 vs 0.395 [IQR: 0.25–1.90], $p > 0.05$, N = 6).

3.3. Humoral response

The median total anti-SARS-CoV-2 antibody concentration of the 34 vaccinated uninfected HCWs increased after the third dose (203 BAU/mL [IQR: 107–343] vs. 2941 BAU/mL [IQR: 1562–3900], $p < 0.0001$) (Fig. 2). The median total anti-SARS-CoV-2 antibody

Table 1
Patient characteristics.

HCWs (N = 40)	Vaccinated uninfected	Vaccinated infected
n (%)	34 (85)	6 (15)
Age: median (IQR)	38 (32.5–43)	46.5 (32.7–54.2)
Female: n (%)	27 (79.4)	4 (66.7)
Immunosuppressive therapy: n (%)	1 (2.9)	1 (16.7)
Vaccines: n (%)	6 (17.6)	1 (16.7)
BNT162b/BNT162b/BNT162b		
BNT162b/BNT162b/mRNA-1273	27 (79.4)	5 (83.3)
mRNA-1273/mRNA-1273/mRNA-1273	1 (3)	0

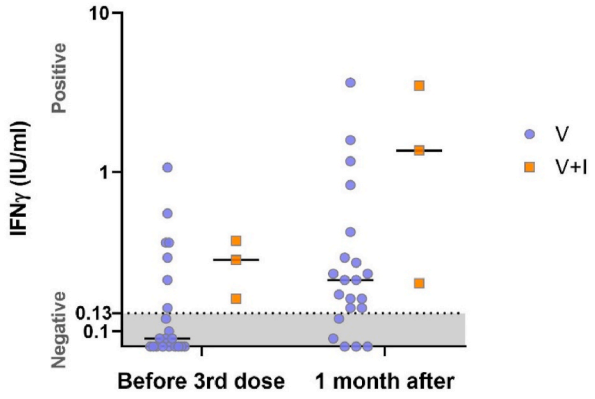


Fig. 1. Concentration of interferon gamma.
Comparison of cellular response, expressed as IFNγ (IU/mL), in vaccinated uninfected (V) (N = 21) and infected HCWs (V + I) (N = 3) before and one month after the third dose vaccine. Horizontal bars indicate the median.

concentration of the 6 vaccinated infected HCWs increased with the third dose (114 BAU/mL [IQR: 81–236] vs. 7114 BAU/mL [IQR: 2991–11,833], $p < 0.05$) and was higher in comparison with the vaccinated uninfected HCWs one month after the third dose vaccine (7114 BAU/mL [IQR: 2991–11,833] vs 2941 BAU/mL [IQR: 1562–3900], $p < 0.05$) (Fig. 2). Before the third dose, no difference was found between the total anti-SARS-CoV-2 antibody concentrations in vaccinated uninfected and infected HCWs (203 BAU/mL [IQR: 107–343] vs. 114 BAU/mL [IQR: 81–235.5], $p > 0.05$).

Neutralizing antibodies were detected in 55.9% (19/34) and in 100% (34/34) of vaccinated uninfected HCWs before and one month after the third dose respectively. Neutralizing antibodies were detected in 16.7 % (1/6) and 100% (6/6) of vaccinated infected HCWs before and after the third dose. The NAb titer increased with the third dose in vaccinated uninfected HCWs (2 [IQR: 0–2] vs. 16 [IQR: 14–32], $p < 0.0001$) and vaccinated infected HCWs (0 [IQR: 0–1] vs. 48 [IQR: 28–100], $p < 0.05$) (Fig. 3). No difference was found between the NAb titer before the third dose in the vaccinated uninfected and infected HCWs (2 [IQR: 0–2] vs. 0 (IQR: 0–1), $p > 0.05$). One month after the third dose, the NAb titer of the vaccinated infected HCWs was higher than that of the vaccinated uninfected HCWs (48 [IQR: 28–160] vs. 16 [IQR: 14–32], $p = 0.01$) (Fig. 3).

The Spearman correlation between the titers of NAb and total anti-SARS-CoV-2 antibodies in vaccinated uninfected HCWs was 0.638 ([95% CI: 0.37–0.81], $p < 0.001$) (Fig. 4A) and 0.782 ([95% CI: 0.60–0.89], $p < 0.001$) (Fig. 4B) before and after the third dose of vaccine. However, no monotonic correlation was found among vaccinated infected HCWs. No Spearman correlations were found between total binding antibodies or NAb and IFNγ release before and after the third dose in vaccinated uninfected HCWs (Supplementary Data).

4. Discussion

This study focused on examining the immune response of HCWs who received a third dose of the mRNA vaccine. We analyzed both cellular and humoral immune responses using whole blood IGRA to measure cellular responses and by determining the levels of anti-

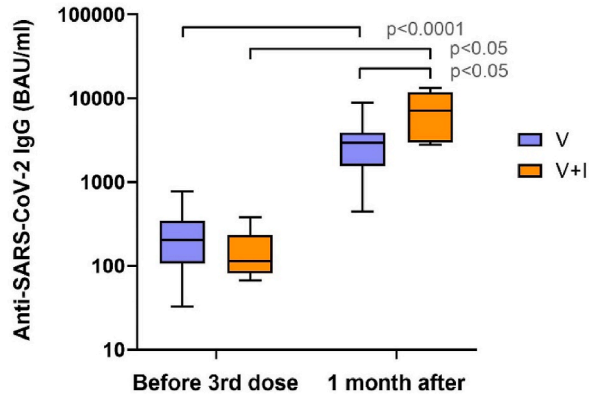


Fig. 2. Total anti-SARS-CoV-2 antibody concentrations.
Comparison of total anti-SARS-CoV-2 IgG serum antibody levels in vaccinated uninfected (V) (N = 34) and infected (V + I) (N = 6) HCWs before and after the third dose of the vaccine. Bars indicate median values, box boundaries represent IQRs, and whiskers indicate the maximum and minimum values.

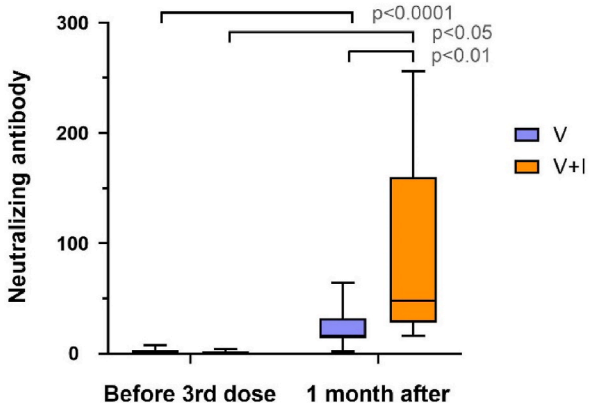


Fig. 3. Neutralizing antibody titers.
Comparison of NAb titers in vaccinated uninfected (V) (N = 34) and infected (V + I) (N = 6) HCWs before and after the third dose of the vaccine. Bars indicate median values, box boundaries represent IQRs, and whiskers indicate the maximum and minimum values.

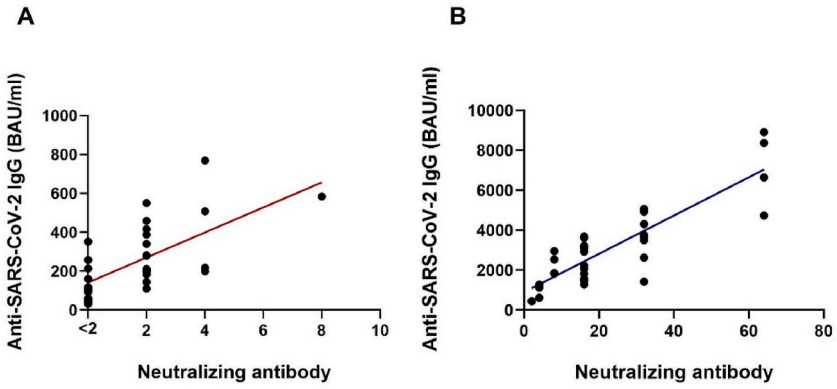


Fig. 4. Neutralizing antibody titers and total anti-SARS-Cov-2 antibody concentrations.
(A) Binding antibody concentrations according to NAb titers among vaccinated uninfected HCWs before the third dose and trend curve (red line). (B) Binding antibody concentrations according to NAb titers among vaccinated uninfected HCWs one month after the third dose and the trend curve (blue line). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

spike antibodies and NAb to assess humoral responses. Our findings indicated that the third dose of the mRNA vaccine significantly enhanced both the cellular and humoral immune responses. The main results of our study showed that total SARS-CoV-2 specific antibodies and NAb levels in vaccinated infected HCWs were higher than those in uninfected HCWs. The cellular immune response, objectified by a positive IGRA result, was not associated with protection against SARS-CoV-2 infection.

Although immunity against SARS-CoV-2 has decreased over time, the most effective method to fight the pandemic is COVID-19 vaccination. Research has documented a decline in antibodies 3–6 months after two doses of the mRNA vaccine in both infected [14,35] and uninfected subjects [12,14,35]. As expected, our subjects exhibited low but detectable levels of anti-SARS-CoV-2 antibodies six months after their second dose. Among the six HCWs infected during the study five did not have NAbs against the Omicron variant prior to the infection. The omicron variant is known for its ability to evade NAb which could explain why the patient was infected despite receiving a two-dose regimen. Our results show that the booster increased the humoral response. Specifically, the administration of the third-dose vaccine substantially enhanced the NAb response to the Omicron variant. The presence of neutralizing antibodies produced by vaccination is crucial because of their protective role against SARS-CoV-2 [35–37]. However, the total and neutralizing antibody levels observed in uninfected HCWs were lower than those observed in infected HCWs. Consistent with the literature [35,38,39], this finding suggests that hybrid immunity generates a stronger immune response than vaccination alone.

Multiple pieces of evidence indicates that cellular responses are crucial for overcoming SARS-CoV-2 infection and COVID-19 [40]. Cellular responses regulate disease severity in humans [35] and lower viral loads in non-human primates [41,42]. After experiencing COVID-19 [43,44] or receiving mRNA vaccines [14], strong and durable memories of $CD4^+$ and $CD8^+$ T cells are generated. Previous studies on long-term immune responses during infection with severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) have demonstrated a detectable memory response of T cells at 17 years post-infection, whereas memory B cells have shown shorter lifespans [45,46]. Primorac et al. demonstrated a long-lasting cellular response, observed up to 20 months after the last contact with the SARS-CoV-2 spike antigen, either through infection or vaccination [47]. We found that six months after completing

the full vaccination schedule, 42.9% of uninfected HCWs had a detectable cellular response. Studies that also used IGRA to assess cellular immunity demonstrated that 19% of individuals produced IFNγ 9 months after receiving two doses [37] and 50% of individuals 6–10.5 months after three doses of BN162b2 vaccine [48]. Our results indicated that the booster dose enhanced the cellular immune response in uninfected HCWs, as evidenced by the increased proportion of positive IGRA results (76.2%). However, we do not show changes in IFNγ concentration among uninfected and infected HCWs, while other studies have shown a stronger cellular response in individuals infected and vaccinated [35,47,49]. The infected HCWs exhibited positive IGRA test results both before and after the third vaccine dose. Despite the small sample size, our results indicated that the cellular immune response does not provide protection against SARS-CoV-2 infection. Furthermore, our observations indicate the heterogeneity of cellular responses. This heterogeneity of responses in infected HCWs could be influenced by the severity of SARS-CoV-2 infection, as studies have shown an association between disease severity and the cellular immune response [50,51].

Consistent with the literature [14,38,52,53], we demonstrated a correlation between NAbs and total anti-SARS-CoV-2 antibody level. However, we did not observe a relationship between the cellular and humoral responses before and after the third vaccine dose. In contrast, Primorac et al. demonstrated a positive correlation between these two elements, regardless of whether the subjects were infected/or vaccinated [47]. Campagna et al. also showed that median antibody levels were higher in the IGRA-positive group than in the IGRA-negative group [54]. These differences could be explained by variations in the sample size, tools used to measure cellular immunity, or vaccination regimens.

Our study had some limitations, including a small sample size and a cohort composed of young HCWs with a median age of 38.5 years, which prevented the generalization of our results to other populations. A larger sample size would provide more robust results and increase the statistical power. Additionally, stimulating T cells from the peripheral circulating blood does not provide insight into the T cell response at the site of infection. Similarly, the IFNγ release assay is a simple measure of cellular immunity but does not distinguish between subgroups of T cells and other IFNγ-producing cells. There is significant variability in the methods used, and direct comparisons of the results of these methods have rarely been performed. It is also impossible to determine whether HCWs who did not show an IFNγ response had a response below the detection limits of the test used or if they truly did not have a cellular immune response. Furthermore, long-term studies are required to estimate the kinetics of the cellular responses to IGRA.

5. Conclusion

This study demonstrated that the third dose of vaccine reinforce both humoral and cellular immune responses. Our results showed that, unlike humoral immunity, the cellular response did not increase in the case of infection. The cellular response was not associated with protection against SARS-CoV-2 infection. The highest levels of anti-spike antibodies and NAbs were found in vaccinated and infected HCWs. Measuring cellular immunity through an IGRA is a simple and suitable routine method to document the cellular response to vaccination. However, harmonization of cellular immune measurement techniques is necessary to compare results across different studies, as well as to establish a threshold that demonstrates that the cellular immune response is adequately elicited to provide protection against the virus. Furthermore, future studies on cellular immunity in other populations, such as immunocompromised patients, are needed to understand the immunity against SARS-CoV-2 and other potential epidemics. In addition, studies on the long-term immune response to a third vaccine dose are necessary. This knowledge is crucial for the development of targeted vaccination strategies, monitoring the effectiveness of vaccines against emerging variants, and controlling the COVID-19 pandemic.

Ethics declaration

Informed consent was not required for this study because biological material and clinical data were obtained only for standard virological monitoring. Data were analyzed using an anonymized database. Such a protocol does not require written informed consent according to French Public Health law (CSP Art L 1121-1.1).

Funding

This work was supported by Toulouse Institute for Infectious and Inflammatory Diseases (Infinity) (INSERM UMR 1291, CNRS UMR 5051, Université Toulouse III, France).

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Faustine Montmaneix-Engels: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Chloé Dimeglio:** Supervision, Methodology, Formal analysis. **Laeticia Staes:** Methodology, Investigation. **Isabelle Da Silva:** Investigation. **Marion Porcheron:** Investigation. **Isabelle Jougla:** Resources. **Fabrice Hérin:** Conceptualization. **Jacques Izopet:** Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank bioMérieux SA for providing reagents. We also thank all the personnel of the Occupational Diseases Department at Purpan Hospital in Toulouse who contributed to the sample collection.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29116.

References

[1] X. Castro Dopico, S. Ols, K. Loré, G.B. Karlsson Hedestam, Immunity to SARS-CoV-2 induced by infection or vaccination, J. Intern. Med. 291 (1) (2022) 32–50, https://doi.org/10.1111/joim.13372.
[2] S.H. Kim, Interferon-γ release assay for cytomegalovirus (IGRA-CMV) for risk stratification of posttransplant CMV infection: is it time to apply IGRA-CMV in routine clinical practice? Clin. Infect. Dis. 71 (9) (2020) 2386–2388, https://doi.org/10.1093/cid/ciz1211.
[3] W. Mouton, C. Compagnon, K. Saker, S. Daniel, S. Djebali, X. Lacoux, et al., Specific detection of memory T-cells in COVID-19 patients using standardized whole-blood Interferon gammarelease assay, Eur. J. Immunol. 51 (12) (2021) 3239–3242, https://doi.org/10.1002/eji.202149296.
[4] L.A. Jackson, E.J. Anderson, N.G. Rouphael, P.C. Roberts, M. Makhene, R.N. Coler, et al., An mRNA vaccine against SARS-CoV-2 - preliminary report, N. Engl. J. Med. 383 (20) (2020) 1920–1931, https://doi.org/10.1056/NEJMoa2022483.
[5] A.B. Vogel, I. Kanevsky, Y. Che, K.A. Swanson, A. Muik, M. Vormehr, et al., BNT162b vaccines protect rhesus macaques from SARS-CoV-2, Nature 592 (7853) (2021) 283–289, https://doi.org/10.1038/s41586-021-03275-y.
[6] Y.N. Lamb, BNT162b2 mRNA COVID-19 vaccine: first approval, Drugs 81 (4) (2021) 495–501, https://doi.org/10.1007/s40265-021-01480-7.
[7] A. Bertoletti, A.T. Tan, N. Le Bert, The T-cell response to SARS-CoV-2: kinetic and quantitative aspects and the case for their protective role, Oxf. Open Immunol. 2 (1) (2021) iqab006, https://doi.org/10.1093/oxfimm/iqab006.
[8] E.G. Levin, Y. Lustig, C. Cohen, R. Fluss, V. Indenbaum, S. Amit, et al., Waning immune humoral response to BNT162b2 covid-19 vaccine over 6 months, N. Engl. J. Med. 385 (24) (2021) e84, https://doi.org/10.1056/NEJMoa2114583.
[9] P.B. Gilbert, D.C. Montefiori, A.B. McDermott, Y. Fong, D. Benkeser, W. Deng, et al., Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial, Science 375 (6576) (2022) 43–50, https://doi.org/10.1126/science.abm3425.
[10] D.S. Khoury, D. Cromer, A. Reynaldi, T.E. Schlub, A.K. Wheatley, J.A. Juno, et al., Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection, Nat. Med. 27 (7) (2021) 1205–1211, https://doi.org/10.1038/s41591-021-01377-8.
[11] S. Cele, L. Jackson, D.S. Khoury, K. Khan, T. Moyo-Gwete, H. Tegally, et al., Omicron extensively but incompletely escapes Pfizer BNT162b2 neutralization, Nature 602 (7898) (2022) 654–656, https://doi.org/10.1038/s41586-021-04387-1.
[12] N. Doria-Rose, M.S. Suthar, M. Makowski, S. O'Connell, A.B. McDermott, B. Flach, et al., Antibody persistence through 6 Months after the second dose of mRNA-1273 vaccine for covid-19, N. Engl. J. Med. 384 (23) (2021) 2259–2261, https://doi.org/10.1056/NEJMc2103916.
[13] D. Planas, D. Veyer, A. Baidaliuk, I. Staropoli, F. Guivel-Benhassine, M.M. Rajah, et al., Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization, Nature 596 (7871) (2021) 276–280, https://doi.org/10.1038/s41586-021-03777-9.
[14] R.R. Goel, M.M. Painter, S.A. Apostolidis, D. Mathew, W. Meng, A.M. Rosenfeld, et al., mRNA vaccines induce durable immune memory to SARS-CoV-2 and variants of concern, Science 374 (6572) (2021) abm0829, https://doi.org/10.1126/science.abm0829.
[15] A. Sette, S. Crotty, Immunological memory to SARS-CoV-2 infection and COVID-19 vaccines, Immunol. Rev. 310 (1) (2022) 27–46, https://doi.org/10.1111/imr.13089.
[16] A. Tarke, C.H. Coelho, Z. Zhang, J.M. Dan, E.D. Yu, N. Methot, et al., SARS-CoV-2 vaccination induces immunological T cell memory able to cross-recognize variants from Alpha to Omicron, Cell 185 (5) (2022), https://doi.org/10.1016/j.cell.2022.01.015, 847-85.e11.
[17] Z. Zhang, J. Mateus, C.H. Coelho, J.M. Dan, C.R. Moderbacher, R.I. Gálvez, et al., Humoral and cellular immune memory to four COVID-19 vaccines, Cell 185 (14) (2022) 2434–2451.e17, https://doi.org/10.1016/j.cell.2022.05.022.
[18] K. Kedzierska, P.G. Thomas, Count on us: T cells in SARS-CoV-2 infection and vaccination, Cell Rep. Med. 3 (3) (2022) 100562, https://doi.org/10.1016/j.xcrm.2022.100562.
[19] J. Mateus, A. Grifoni, A. Tarke, J. Sidney, S.I. Ramirez, J.M. Dan, et al., Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans, Science 370 (6512) (2020) 89–94, https://doi.org/10.1126/science.abd3871.
[20] J. Liu, A. Chandrashekar, D. Sellers, J. Barrett, C. Jacob-Dolan, M. Lifton, et al., Vaccines elicit highly conserved cellular immunity to SARS-CoV-2 Omicron, Nature 603 (7901) (2022) 493–496, https://doi.org/10.1038/s41586-022-04465-y.
[21] Y. Gao, C. Cai, A. Grifoni, T.R. Müller, J. Niessl, A. Olofsson, et al., Ancestral SARS-CoV-2-specific T cells cross-recognize the Omicron variant, Nat. Med. 28 (3) (2022) 472–476, https://doi.org/10.1038/s41591-022-01700-x.
[22] D. Goldblatt, G. Alter, S. Crotty, S.A. Plotkin, Correlates of protection against SARS-CoV-2 infection and COVID-19 disease, Immunol. Rev. 310 (1) (2022) 6–26, https://doi.org/10.1111/imr.13091.
[23] A. Tarke, J. Sidney, N. Methot, E.D. Yu, Y. Zhang, J.M. Dan, 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. 2 (7) (2021) 100355, https://doi.org/10.1016/j.xcrm.2021.100355.
[24] P. Danza, T.H. Koo, M. Haddix, R. Fisher, E. Traub, K. Oyong, et al., SARS-CoV-2 infection and hospitalization among adults aged ≥18 Years, by vaccination status, before and during SARS-CoV-2 B.1.1.529 (omicron) variant predominance - Los Angeles county, California, november 7, 2021-january 8, 2022, MMWR Morb. Mortal. Wkly. Rep. 71 (5) (2022) 177–181, https://doi.org/10.15585/mmwr.mm7105e1.
[25] H.N. Altarawneh, H. Chemaitelly, M.R. Hasan, H.H. Ayoub, S. Qassim, S. AlMukdad, et al., Protection against the omicron variant from previous SARS-CoV-2 infection, N. Engl. J. Med. 386 (13) (2022) 1288–1290, https://doi.org/10.1056/NEJMc2200133.
[26] M. Schwarz, S. Mzoughi, D. Lozano-Ojalvo, A.T. Tan, A. Bertoletti, E. Guccione, T cell immunity is key to the pandemic endgame: how to measure and monitor it, Curr. Res. Immunol. 3 (2022) 215–221, https://doi.org/10.1016/j.crimmu.2022.08.004.
[27] M. Pai, M. Behr, Latent Mycobacterium tuberculosis infection and interferon-gamma release assays, Microbiol. Spectr. 4 (5) (2016), https://doi.org/10.1128/microbiolspec.TBTB2-0023-2016.

[28] Y. Renaudineau, F. Abravanel, J. Izopet, C. Bost, E. Treiner, N. Congy, et al., Novel T cell interferon gamma release assay (IGRA) using spike recombinant protein for COVID19 vaccine response and Nucleocapsid for SARS-Cov2 response, Clin. Immunol. 237 (2022) 108979, https://doi.org/10.1016/j.clim.2022.108979.
[29] M. Crespo, A. Barrilado-Jackson, E. Padilla, J. Eguía, D. Echeverria-Esnal, H. Cao, et al., Negative immune responses to two-dose mRNA COVID-19 vaccines in renal allograft recipients assessed with simple antibody and interferon gamma release assay cellular monitoring, Am. J. Transplant. 22 (3) (2022) 786–800, https://doi.org/10.1111/ajt.16854.
[30] B. Pozzetto, V. Legros, S. Djebali, V. Barateau, N. Guibert, M. Villard, et al., Immunogenicity and efficacy of heterologous ChAdOx1-BNT162b2 vaccination, Nature 600 (7890) (2021) 701–706, https://doi.org/10.1038/s41586-021-04120-y.
[31] C. Dimeglio, F. Herin, I. Da-Silva, C. Gernigon, M. Porcheron, S. Chapuy-Regaud, et al., Decreased efficiency of neutralizing antibodies from previously infected or vaccinated individuals against the B.1.617.2 (delta) SARS-CoV-2 variant, Microbiol. Spectr. 10 (4) (2022) e0270621, https://doi.org/10.1128/spectrum.02706-21.
[32] C. Dimeglio, M. Migueres, N. Bouzid, S. Chapuy-Regaud, C. Gernigon, I. Da-Silva, et al., Antibody titers and protection against omicron (BA.1 and BA.2) SARS-CoV-2 infection, Vaccines 10 (9) (2022) 1548, https://doi.org/10.3390/vaccines10091548.
[33] S. Chapuy-Regaud, M. Miédougé, F. Abravanel, I. Da Silva, M. Porcheron, J. Fillaux, et al., Evaluation of three quantitative anti-SARS-CoV-2 antibody immunoassays, Microbiol. Spectr. 9 (3) (2021) e0137621, https://doi.org/10.1128/spectrum.01376-21.
[34] C. Dimeglio, I. Da-Silva, M. Porcheron, M.P. Panero, L. Staes, P. Trémeaux, et al., Weaker effects of the fourth dose of BNT162b2 SARS-CoV-2 vaccine on the elderly human population, Vaccines 11 (6) (2023) 1095, https://doi.org/10.3390/vaccines11061095.
[35] M. Chivu-Economescu, C. Bleotu, C. Grancea, D. Chiriac, A. Botezatu, I.V. Iancu, et al., Kinetics and persistence of cellular and humoral immune responses to SARS-CoV-2 vaccine in healthcare workers with or without prior COVID-19, J. Cell Mol. Med. 26 (4) (2022) 1293–1305, https://doi.org/10.1111/jcmm.17186.
[36] F. Petrelli, A. Luciani, K. Borgonovo, M. Ghilardi, M.C. Parati, D. Petrò, et al., Third dose of SARS-CoV-2 vaccine: a systematic review of 30 published studies, J. Med. Virol. 94 (6) (2022) 2837–2844, https://doi.org/10.1002/jmv.27644.
[37] R. Busà, M.C. Sorrentino, G. Russelli, G. Amico, V. Miceli, M. Miele, et al., Specific anti-SARS-CoV-2 humoral and cellular immune responses after booster dose of BNT162b2 pfizer-BioNTech mRNA-based vaccine: integrated study of adaptive immune system components, Front. Immunol. 13 (2022) 856657, https://doi.org/10.3389/fimmu.2022.856657.
[38] T.A. Bates, S.K. McBride, H.C. Leier, G. Guzman, Z.L. Lyski, D. Schoen, et al., Vaccination before or after SARS-CoV-2 infection leads to robust humoral response and antibodies that effectively neutralize variants, Sci. Immunol. 7 (68) (2022) eabn8014, https://doi.org/10.1126/sciimmunol.abn8014.
[39] C. Dimeglio, M. Migueres, S. Chapuy-Regaud, I. Da-Silva, I. Jougla, C. Pradere, et al., Comparative effects of mRNA vaccine booster and natural Omicron infection on the neutralizing antibody response, J. Infect. 85 (1) (2022) e4–e6, https://doi.org/10.1016/j.jinf.2022.05.001.
[40] A. Sette, S. Crotty, Adaptive immunity to SARS-CoV-2 and COVID-19, Cell 184 (4) (2021) 861–880, https://doi.org/10.1016/j.cell.2021.01.007.
[41] K. McMahan, J. Yu, N.B. Mercado, C. Loos, L.H. Tostanoski, A. Chandrashekar, et al., Correlates of protection against SARS-CoV-2 in rhesus macaques, Nature 590 (7847) (2021) 630–634, https://doi.org/10.1038/s41586-020-03041-6.
[42] A. Chandrashekar, J. Liu, A.J. Martinot, K. McMahan, N.B. Mercado, L. Peter, et al., SARS-CoV-2 infection protects against rechallenge in rhesus macaques, Science 369 (6505) (2020) 812–817, https://doi.org/10.1126/science.abc4776.
[43] J. Zuo, A.C. Dowell, H. Pearce, K. Verma, H.M. Long, J. Begum, et al., Robust SARS-CoV-2-specific T cell immunity is maintained at 6 months following primary infection, Nat. Immunol. 22 (5) (2021) 620–626, https://doi.org/10.1038/s41590-021-00902-8.
[44] J.M. Dan, J. Mateus, Y. Kato, K.M. Hastie, E.D. Yu, C.E. Faliti, et al., Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection, Science 371 (6529) (2021) eabf4063, https://doi.org/10.1126/science.abf4063.
[45] N. Le Bert, A.T. Tan, K. Kunasegaran, C.Y.L. Tham, M. Hafezi, A. Chia, et al., SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls, Nature 584 (7821) (2020) 457–462, https://doi.org/10.1038/s41586-020-2550-z.
[46] L.P. Wu, N.C. Wang, Y.H. Chang, X.Y. Tian, D.Y. Na, L.Y. Zhang, et al., Duration of antibody responses after severe acute respiratory syndrome, Emerg. Infect. Dis. 13 (10) (2007) 1562–1564, https://doi.org/10.3201/eid1310.070576.
[47] D. Primorac, P. Brlek, V. Matišić, V. Molnar, K. Vrdoljak, R. Zadro, et al., Cellular immunity-the key to long-term protection in individuals recovered from SARS-CoV-2 and after vaccination, Vaccines 10 (3) (2022) 442, https://doi.org/10.3390/vaccines10030442, n.d.
[48] M.M. Dourdouna, E.B. Tatsi, V. Syriopoulou, A. Michos, Evaluation of T cell responses with the QuantiFERON SARS-CoV-2 assay in individuals with 3 doses of BNT162b2 vaccine, SARS-CoV-2 infection, or hybrid immunity, Diagn. Microbiol. Infect. Dis. 106 (3) (2023) 115948.
[49] V. Naranbhai, A. Nathan, C. Kaseke, C. Berrios, A. Khatri, S. Choi, et al., T cell reactivity to the SARS-CoV-2 Omicron variant is preserved in most but not all individuals, Cell 185 (6) (2022) 1041–1051.e6, https://doi.org/10.1016/j.cell.2022.01.029.
[50] A.T. Tan, M. Linster, C.W. Tan, N. Le Bert, W.N. Chia, K. Kunasegaran, et al., Early induction of functional SARS-CoV-2-specific T cells associates with rapid viral clearance and mild disease in COVID-19 patients, Cell Rep. 34 (6) (2021) 108728, https://doi.org/10.1016/j.celrep.2021.108728.
[51] T. Sekine, A. Perez-Potti, O. Rivera-Ballesteros, K. Strålin, J.B. Gorin, A. Olsson, et al., Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19, Cell 183 (1) (2020) 158–168.e14, https://doi.org/10.1016/j.cell.2020.08.017.
[52] F. Gallais, P. Gantner, T. Bruel, A. Velay, D. Planas, M.J. Wendling, et al., Evolution of antibody responses up to 13 months after SARS-CoV-2 infection and risk of reinfection, EBioMedicine 71 (2021) 103561, https://doi.org/10.1016/j.ebiom.2021.103561.
[53] M. Prendecki, C. Clarke, J. Brown, A. Cox, S. Gleeson, M. Guckian, et al., Effect of previous SARS-CoV-2 infection on humoral and T-cell responses to single-dose BNT162b2 vaccine, Lancet 397 (10280) (2021) 1178–1181, https://doi.org/10.1016/S0140-6736(21)00502-X.
[54] R. Campagna, L. Mazzuti, G. Guerrizio, C. Nonne, G. Migliara, C. De Vito, et al., Humoral and T-cell mediated response after administration of mRNA vaccine BNT162b2 in frail populations, Vaccine X 12 (2022) 100246, https://doi.org/10.1016/j.jvacx.2022.100246.