Journal of Virological Methods 327 (2024) 114949

Contents lists available at ScienceDirect

Journal of Virological Methods

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

SARS-CoV-2-specific T cell responses: a comparative analysis between QuantiFERON SARS-CoV-2, T-SPOT.*COVID*, and an in-house Omicron ELISpot

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ARTICLE INFO

Keywords:
SARS-CoV-2
COVID-19
T cells
IGRA
ELISpot
Immunity

ABSTRACT

Background: T cell immunity plays a pivotal role in mitigating the severity of coronavirus disease 2019 (COVID-19). Therefore, reliable functional T cell assays are required to evaluate severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific T cell immunity in specific patient populations.
Methods: We recruited a cohort of 23 healthcare workers who received their bivalent Omicron BA.1 / ancestral mRNA booster vaccination or were infected with the Omicron variant at a median of 144 days and 227 days before blood collection, respectively. In this cohort, we compared the performances of two widely utilized commercial SARS-CoV-2 interferon-gamma release assays (IGRAs), i.e., QuantiFERON SARS-CoV-2 and T-SPOT.*COVID*, and an in-house designed Omicron enzyme-linked immunospot (ELISpot).
Results: The QuantiFERON SARS-CoV-2 and T-SPOT.*COVID* assays detected SARS-CoV-2 spike-specific T cells in 34.8 % and 21.7 % of participants, respectively. Moreover, our in-house designed ELISpot that included Omicron BA.4 and BA.5 full-spike peptides detected T cell responses in 47.8 % of participants and was strongly associated with the T-SPOT.*COVID*.
Conclusion: The evaluation of SARS-CoV-2 T cell immunity using commercially accessible assays may yield disparate outcomes as results from different assays are not directly comparable. A specific Omicron ELISpot should be considered to assess Omicron-specific T cell immunity.

1. Introduction

As of November 2023, the Omicron variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to exert a significant impact, with more than 95,000 hospitalizations, 1600 intensive care unit admissions, and 4700 deaths per month (COVID-19 weekly epidemiological update 160, 2023). The persistence of hospitalizations, despite prior coronavirus disease 2019 (COVID-19) vaccinations and SARS-CoV-2 infections, can be attributed to the waning of adaptive immunity and the emergence of novel Omicron subvariants (Carabelli et al., 2023; Xia et al., 2022).

Numerous studies have consistently empphasized the crucial role of T cells in conferring protective immunity against COVID-19 (Almendro-Vázquez et al., 2023; Moss, 2022). Notably, T cells possess a unique advantage compared to their humoral counterparts as they exhibit cross-reactivity against various SARS-CoV-2 variants, allowing for cellular defense against new SARS-CoV-2 variants (Moss, 2022). Consequently, it remains important to also reliably monitor SARS-CoV-2-specific T cell immunity in addition to antibody responses. This practice is particularly relevant for patient populations with an elevated risk of developing severe manifestations of COVID-19.

In diagnostic laboratories, the evaluation of T cell immunity is commonly conducted by detecting interferon-gamma (IFN-γ) secretion subsequent to T cell stimulation with specific antigens. This assessment method is known as IFN-γ release assays (IGRAs). The detection of antigen-specific IFN-γ responses provides an indication of T cell protection against SARS-CoV-2 since IFN-γ is primarily produced by $CD4^+$ T helper 1 cells and $CD8^+$ cytotoxic T cells. These T cell subsets support the

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https://doi.org/10.1016/j.jviromet.2024.114949
Received 7 November 2023; Received in revised form 25 April 2024; Accepted 1 May 2024
Available online 6 May 2024

overall adaptive immune response and kill virus-infected cells, respectively (Calarota and Baldanti, 2013).

Several studies have compared the performances of commercially available and in-house developed SARS-CoV-2 IGRAs (Aiello et al., 2022; Jang et al., 2023; Johnson et al., 2023; Mak et al., 2022a; Phillips et al., 2022; Widyasari et al., 2022). However, direct comparisons between the widely used QuantiFERON SARS-CoV-2 and T-SPOT.*COVID* IGRAs are limited (Seo et al., 2023). Moreover, these commercial assays typically utilize antigens from the original Wuhan strain of SARS-CoV-2 while the Omicron variant has emerged as the dominant strain worldwide (COVID-19 weekly epidemiological update 160, 2023). Therefore, this study aimed to compare the performances of the two widely used commercial T cell assays and an in-house developed Omicron ELISpot.

2. Methods

2.1. Study overview

This study included 23 healthcare workers (HCWs) who participated in an ongoing cohort study (Faas et al., 2022). For this study blood samples were collected in heparin tubes via venipuncture in March 2023. Subsequently, these samples were analyzed by three different IGRA assays. This study received approval from the Medical Research Ethical Committee United (protocol number R20.030) and was conducted following the principles of the Declaration of Helsinki.

2.2. QuantiFERON SARS-CoV-2

The QuantiFERON SARS-CoV-2 (Qiagen, Germany) was performed according to the manufacturer's instructions. First, 1 mL heparinized blood was incubated in Ag1 (i.e., CD4 spike S1 peptide pool), Ag2 (i.e., CD4 and CD8 spike S1+S2 peptide pool), mitogen (positive control), and Nil (negative control) blood collection tubes for 16–24 h at 37°C. The plasma was harvested and stored at −20°C until performing the IFN-γ enzyme-linked immunosorbent assay (ELISA), which was performed by a fully automated microplate processor (ETI-MAX, Diasorin, Italy). A 4-point IFN-γ standard (i.e., 4.0, 1.0, 0.25, and 0 IU/mL) was created and 50 μl of each standard or plasma sample was added in separate wells. Next, 50 μl of conjugate was added to all wells and was incubated for 2 h at room temperature (RT). After washing the wells with wash buffer, 100 μl substrate was added and incubated for 30 min, whereafter the reaction was stopped with 50 μl stop solution. The optical density was measured at 450 nm and 620 nm as reference. The IFN-γ concentrations of the plasma samples were calculated from the standard curve, whereafter the negative control was subtracted from the samples. Samples with an IFN-γ concentration of ≥0.15 IU/mL were considered positive.

2.3. T-SPOT.COVID

The T-SPOT.*COVID* (Oxford Immunotec, UK) ELISpot assay was performed according to the manufacturer's instructions. Peripheral blood mononuclear cells (PBMCs) were isolated from blood and were incubated for 16–20 h per 2.5 × 10^5 PBMCs in AIM-V medium with phytohemagglutinin as a positive control, SARS-CoV-2 spike subunit 1 (S1) peptides, or without stimulants as negative control. Spike S1 peptides that were homologous to endemic coronavirus were removed from the pool by the manufacturer. Secreted IFN-γ was captured by an anti-IFN-γ antibody precoated on polyvinylidene fluoride (PVDF) membranes, which was subsequently visualized using an alkaline phosphatase-conjugated and substrate.

2.4. In-house Omicron ELISpot

The in-house Omicron ELISpot was performed as previously described for the SARS-CoV-2 variant ELISpot (Faas et al., 2022). In short, PBMCs were isolated from blood and were incubated for 16–20 h per 2.5 × 10^5 PBMCs in AIM-V medium with anti-CD3 monoclonal antibody (1:1000, mAb CD3-2, Mabtech, Sweden) as positive control, Omicron BA.4/ BA.5 spike peptide pool (SARS-CoV-2 Spike B.1.1.529 / BA.4 & BA.5 / Omicron, PepMix, Germany), or without stimulants as negative control. This Omicron BA.4/BA.5 peptide pool consisted of 158 BA.4 and 157 BA.5 peptides, which were diluted in dimethyl sulfoxide (DMSO) and AIM-V medium to a final concentration of 0.66 μg/mL. Secreted IFN-γ was captured by an anti-IFN-γ monoclonal antibody (mAb 1-D1K; Mabtech) that was precoated on PVDF membranes. Captured IFN-γ was visualized using an alkaline phosphatase-conjugated antibody (1:200, 7-B6-1-ALP; Mabtech) and substrate (BCIPNBT-plus; Mabtech).

Table 1
Characteristics of included antigens in QuantiFERON, T-SPOT.COVID, and in-house Omicron ELISpot assays.

	QuantiFERON Ag1	QuantiFERON Ag2	T-SPOT. *COVID*	In-house Omicron ELISpot
Antigen	WT Spike S1	WT Spike S1+S2	WT Spike S1	Omicron BA.4 and BA.5 Spike S1+S2
Peptide type	Unknown	Unknown	15-mer peptides, 11 aa overlap	15-mer peptides, 11 aa overlap
Reactivity	$CD4^+$ T cells	$CD4^+$ and $CD8^+$ T cells	$CD4^+$ and $CD8^+$ T cells	$CD4^+$ and $CD8^+$ T cells

2.5. ELISpot spot quantification

Spot detection and quantification was applied for the T-SPOT.*COVID* and Omicron ELISpot as described previously (Mak et al., 2022b). In short, spots were visualised with a digital microscope (DX1; Veho®), whereafter images were analysed using the open-source FIJI software. An intensity threshold of 95 was applied instead of 75 to allow for more sensitive spot detection. Reactive T cells were presented as spot-forming cells (SFCs) per well (i.e., per 2.5 × 10^5 PBMCs). Samples were categorized as negative (<5 spots), borderline (5–7 spots), or positive (≥8 spots) as described by the manufacturer for the T-SPOT.*COVID* (T-SPOT.COVID, Oxford Immunotec, 2023) (Table 1).

2.6. Statistical analyses

Data were expressed as median with interquartile range (IQR) and statistical tests were performed at a two-tailed α- level of 0.05 using Graphpad Prism v9 (Graphpad software). The Mann–Whitney U test was used to compare two datasets, whereas the Spearman's rank correlation coefficients were calculated to assess associations between results.

3. Results

Among the 23 HCWs included in this study, their vaccination history indicated a total of two (n=2), three (n=5), four (n=12), or five (n=4) vaccinations, of whom 16 received an Omicron BA.1 / ancestral bivalent mRNA vaccination at a median of 144 (IQR 119–157) days before blood collection. 18 HCWs had experienced previous SARS-CoV-2 infections median 227 (IQR 161–365) days before blood collection, of whom 16 HCWs were infected when Omicron was the predominant variant in the Netherlands (National Institute for Public Health and the Environment (RIVM), 2024).

First, we compared the qualitative and quantitative results after QuantiFERON Ag1 and Ag2 stimulation. At least one of these measurements was positive, indicating the presence of SARS-CoV-2-specific T cell responses, in 34.8 % of HCWs and we observed no significant

Table 2
Qualitative and quantitative results of three different IGRAs (n=23).

	QuantiFERON Ag1	QuantiFERON Ag2	QuantiFERON Ag1 or Ag2	T-SPOT.*COVID*	In-house Omicron ELISpot
Positive (%)	7 (30.4)	7 (30.4)	8 (34.8)	5 (21.7)	11* (47.8)
Borderline (%)	0 (0)	0 (0)	-	5 (21.7)	4* (17.4)
Median response** (IQR)	0.049 (0.024 – 0.255)	0.065 (0.031 – 0.299)	-	4 (1.5 – 6)	7 (4 – 14.5)

* The cut-off was based on the cut-off described for the T-SPOT.*COVID*.
** QuantiFERON responses are presented as IFN-γ IU/mL, whereas T.SPOT.*COVID* and Omicron ELISpot responses are presented as the number of SFCs per well

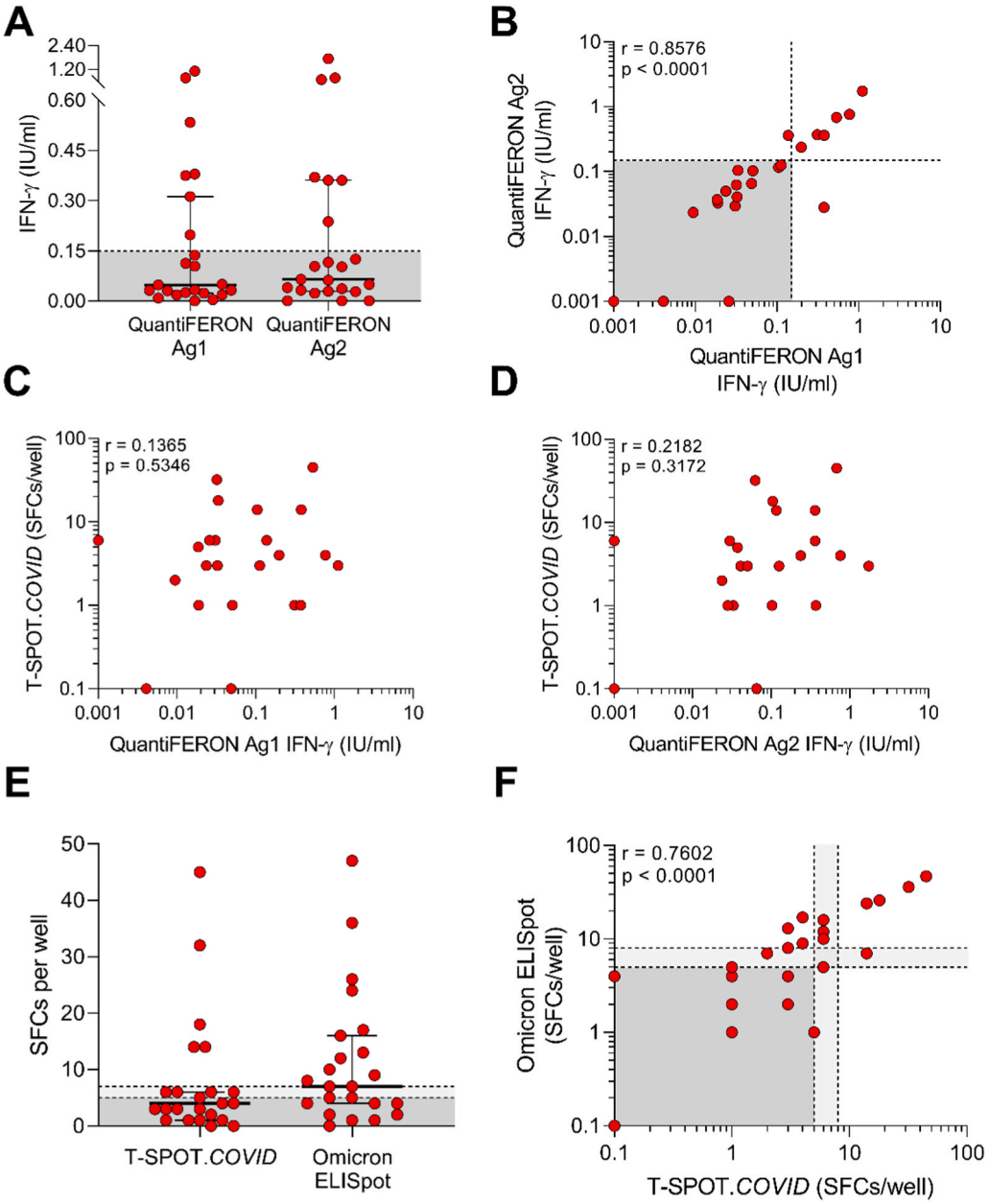

Fig. 1. SARS-CoV-2-specific IFN-γ T cell responses assessed by three IGRAs. (A) Comparison and (B) association between IFN-γ concentrations of QuantiFERON Ag1 and Ag2. Associations between the T-SPOT.*COVID* and QuantiFERON (C) Ag1 and (D) Ag2. (E) Comparison and (B) association between the observed SFCs using the T-SPOT.*COVID* and in-house Omicron ELISpot. (A, B, E, F) Dashed lines indicate negative/positive cut-off. (A, E) Data are presented as median + IQR and significance was tested by a Mann-Whitney U test. (B-D, F) Associations were assessed by Spearman's rank correlation coefficient (r).

differences in IFN-γ concentrations between Ag1 and Ag2 conditions (Table 2, Fig. 1**A**). Moreover, there was a strong correlation of IFN-γ responses after Ag1 and Ag2 stimulations (Fig. 1**B**). Subsequently, we employed the T-SPOT.*COVID* assay and identified spike S1-specific T cell responses in 21.7 % of HCWs. Comparing the outcomes of the two commercial assays, we observed a non-significant weak association between the number of T-SPOT.*COVID* S1-specific T cells and IFN-γ concentrations after both QuantiFERON Ag1 and Ag2 stimulation (Fig. 1**C-D**).

Furthermore, we assessed the performances of our in-house designed

Omicron ELISpot, which included full-spike peptides from the Omicron variant. We observed that 47.8 % of HCWs tested positive for this assay by applying similar cut-off criteria as described for the T-SPOT.*COVID*. There was a strong correlation between the numbers of reactive T cells detected by the commercial T-SPOT.*COVID* and the in-house Omicron assay (Fig. 1**E**, Fig. 1**F**).

4. Discussion

In this study we observed that the in-house developed Omicron ELISpot detected SARS-CoV-2 T cell responses in a larger number of HCWs compared to the commercial IGRAs. Furthermore, the measurements of T cell responses directed against SARS-CoV-2 spike obtained through two commonly used commercial IGRAs, i.e., QuantiFERON SARS-CoV-2 and T-SPOT.*COVID*, exhibited no significant correlation. Notably, only the T-SPOT.*COVID* assay showed a strong positive correlation with our in-house designed Omicron ELISpot.

The QuantiFERON Ag1 measurement targets S1-specific $CD4^+$ T cells, whereas Ag2 targets both S1 and S2-specific $CD4^+$ and $CD8^+$ T cells (Lamara Mahammed et al., 2023). Since our HCW cohort was vaccinated with COVID-19 mRNA vaccines containing full-spike mRNA (Heinz and Stiasny, 2021; Rijkers et al., 2021), higher responses were expected for Ag2. However, both our study and previous studies have observed comparable responses between Ag1 and Ag2 after COVID-19 vaccination (Aiello et al., 2022; Jaganathan et al., 2021; Jang et al., 2023; Johnson et al., 2023).

The observed differences between the QuantiFERON and T-SPOT.*COVID* assays can likely be attributed by the specific incorporated spike antigens and assay methodology. In more detail, the T-SPOT.*COVID* only detects S1-specific $CD4^+$ and $CD8^+$ T cells and excludes peptide sequences homologous to common cold coronaviruses (CCC) (T-SPOT.COVID, Oxford Immunotec, 2023), possibly explaining why less HCWs tested positive with this assay. Furthermore, the T-SPOT.*COVID* assay quantifies the number of IFN-γ-producing antigen-specific T cells as its output, whereas the QuantiFERON assay measures the total IFN-γ production. The distinction in these output metrics potentially explains why we did not found an association between these assays since the IFN-γ production may vary between different reactive T cell subsets and individuals (Moss, 2022).

Finally, we developed and tested the Omicron-specific ELISpot assay since the assessment of Omicron-specific T cell responses may be most relevant to predict protective immunity during the Omicron era. This in-house ELISpotdetected T cell responses in more HCWs than the commercial assays. However, it should be noted that a similar cut-off was applied for the in house assay and T-SPOT.*COVID*, whereas these assays include different antigens. The inclusion of peptides that cover the full Spike protein of both Omicron BA.4 and BA.5 in the in-house assay may have captured a broader range of T cell responses. Additionally, the lack of exclusion of homologous peptides related to CCC in our in-house assay might have allowed for cross-reactive T cell responses against these CCC (Tarke et al., 2023). Furthermore, the strong association between the T-SPOT.*COVID* and an in-house Omicron ELISpot was also confirmed in a previous study (Seo et al., 2023), and can be explained by the preservation of T cell epitopes among SARS-CoV-2 variants and the ability of T cells to cross-recognize mutated epitopes (Meyer et al., 2023; Naranbhai et al., 2022).

The present study is one of the first to compare the QuantiFERON SARS-CoV-2, T-SPOT.*COVID*, and an in-house Omicron IGRA. However, the study is limited by its small size and the lack of a control group of individuals who have not been exposed to SARS-CoV-2 spike antigens by SARS-CoV-2 infection or COVID-19 vaccination. Ideally, our findings remain to be validated in a larger cohort including groups of 'unexposed', recently SARS-CoV-2 Omicron infected, COVID-19 vaccinated, and both infected and vaccinated individuals.

In conclusion, the evaluation of T cell immunity through the utilization of commercially accessible assays such as the QuantiFERON SARS-CoV-2 or T-SPOT.*COVID* may yield disparate outcomes. Therefore, cautiousness is warrented in the interpretation of measured T cell responses as results may largely depend on the type of IGRA used. Our in-house developed SARS-CoV-2 ELISpot assay that includes full-spike peptides from the Omicron variant might generate the highest positivity rates.

Funding

The study received no funding.

CRediT authorship contribution statement

Johannes G. M. Koeleman: Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **David S. Y. Ong:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Willem A. Mak:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Wendy Visser:** Data curation, Formal analysis, Writing – review & editing.

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.

Data availability

Data will be made available on request.

Acknowledgments

We thank our research nurse Marijke van der Vliet for her assistance in this prospective cohort study, and our technicians Nicolette van Drunen, Joyce van Veen, and Frans Keuren for their assistance during the laboratory tests.

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