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Comparison between an in-house SARS-CoV-2 ELISpot and the T-Spot® Discovery SARS-CoV-2 for the assessment of T cell responses in prior SARS-CoV-2-infected individuals

Adaptive immune responses induced by SARS-CoV-2 vaccination or infection decrease over time and, especially neutralizing antibodies, are less effective against new SARS-CoV-2 variants of concern. [1–5] As immune protection is also determined by the cellular immunity, it is crucial to have T cell assays that can adequately assess SARS-CoV-2-specific T cell responses.

In our previous work, we assessed SARS-CoV-2 antigen-specific T cell responses after SARS-CoV-2 infection and vaccination using the T-Spot® Discovery SARS-CoV-2 kit from Oxford Immunotec. [6] Although the T-Spot® Discovery is a commercial kit that has been extensively used in clinical studies, this kit might not be economically feasible for all laboratories and adaptations to the assay are not possible, such as using different antigen peptide pools containing virus mutations. Therefore, we also developed an in-house SARS-CoV-2 enzyme-linked immunospot (ELISpot) assay. [7] In the current analysis, we compared the performances of our in-house SARS-CoV-2 ELISpot to the T-Spot® Discovery SARS-CoV-2.

Both assays detect SARS-CoV-2 antigen-specific interferon-gamma (IFN-γ)-secreting T cells, which include predominantly CD4+ T helper type 1 (Th1) cells and CD8+ cytotoxic T cells that are crucial for an antiviral immune response. [8–10] Also, peripheral blood mononuclear cells (PBMCs) are stimulated with overlapping peptide pools of SARS-CoV-2 spike subunit 1 (S1), nucleocapsid protein (N), and membrane protein (M) in these assays. The T-Spot® Discovery excludes S1, N, and M peptides that are homologous to endemic coronaviruses. [6] In contrast, our in-house ELISpot does not exclude sequences homologous to endemic coronaviruses. [7]

We determined T cell responses of 90 blood samples collected from 55 healthcare workers who tested SARS-CoV-2 positive 12 months before the first blood collection (Fig. 1). As described in our previous study, blood was collected either before (n = 32) or after (n = 58) receiving the first and second COVID-19 vaccination. [6] T cell responses were not statistically different between both assays after S1 and N stimulation but was significantly higher in the in-house ELISpot after M stimulation (P = 0.04). Furthermore, we found a strong association between S1 responses (r = 0.85) but only moderate associations of N and M responses between both assays (r = 0.43 and r = 0.56, respectively).

SARS-CoV-2 S1 is considered the most prominent target for achieving protective immunity and is thus solely integrated in most SARS-CoV-2 vaccines. [12, 13] Therefore, evaluating the T cell response against S1 is most valuable. S1 responses between both assays were strongly associated. Unlike S1, the N and M proteins of SARS-CoV-2 are highly homologous to proteins in endemic coronaviruses. [14, 15] The observed larger inter-assay differences in N and M responses might be attributable to considerable homologous sequences being removed from the T-Spot® Discovery N and M peptide pools, whereas for the in-house assay no homologous sequences were removed.

In conclusion, we showed that our in-house ELISpot assay was highly correlated with the commercially available T-Spot® Discovery for the assessment of T cell responses against SARS-CoV-2 S1. A great advantage of using an in-house ELISpot is the possibility to easily adapt the S1 peptide pools to more accurately assess specific T cell responses against current circulating viruses (e.g., Omicron (B.1.1.529) variant) and future SARS-CoV-2 variants of concern.
Fig. 1. Comparison between the in-house SARS-CoV-2 ELISpot and T-Spot® Discovery SARS-CoV-2. A total of 90 samples were included from our study cohort (n = 55) that tested SARS-CoV-2 positive 12 months before the first blood collection. The healthcare workers provided a blood sample once (n = 30), twice (n = 15), or thrice (n = 10), of which 32 samples were collected before and 58 samples were collected median 18 (IQR 14–69) days after receiving the first or second COVID-19 vaccination. PBMCs were stimulated for 16–20 h with SARS-CoV-2 antigens in both assays. (A) Total magnitude of IFN-γ responses to tested antigens of in-house ELISpot (red) and T-Spot® Discovery (blue). Datasets were compared with a Mann-Whitney U test. (B) Associations between antigen-specific responses assessed by Spearman’s rank correlation coefficient (r).

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