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Cellular response to Spike antigens in seronegative kidney transplant recipients after SARS-CoV-2 vaccination

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INTRODUCTION

The quality of immune response has been found to be a crucial factor in infection control. Antigen-specific polyfunctional T-cells (e.g., secreting multiple cytokines simultaneously) have been shown to be associated with lower viral loads in chronic infections. Polyfunctional T-cells are also commonly used to assess vaccine-induced immunogenicity. In addition to neutralizing antibodies, protective immunity to SARS-CoV-2 depends on virus-specific T-cells as they are important actors to help in the generation of high-affinity antibodies and in the elimination of virus-infected cells, therefore globally limiting viral replication.

Immunocompromised individuals are especially vulnerable to COVID-19, with a higher risk of developing severe forms and have therefore been considered as the priorities for immunization against SARS-CoV-2. However, there is increasing evidence that vaccine efficacy is lower in immunosuppression clinical settings. Kidney transplant recipients (KTR) are an example of such a context as they are chronically treated with immunosuppressants in order to prevent the acute transplant rejection. Previous reports show that after COVID-19 vaccination, KTR develop a less robust (or even absent, non-responders) antibody response compared to healthy immunocompetent individuals. COVID-19 vaccination of healthy individuals stimulates both humoral and cell-mediated immunity.

Few data are currently available on the concomitant evaluation of humoral and cellular immune responses after COVID-19 vaccination in KTR, compared to immunocompetent subjects. Specially, most of the literature data on cellular responses are based on interferon-gamma releasing assays (IGRA) or activation-induced methods only, with no information available on polyfunctional anti-SARS-CoV-2 T-cells.

Vaccinated non-responders KTR are candidates to prophylactically receive neutralizing monoclonal antibodies directed against the Spike protein. Although not yet reaching a national consensus, a possible clinical strategy is to administer these antibodies only on non-responders KTR in which no concomitant cellular responses to vaccine antigen is detected. By using multiparametric flow cytometry, we then evaluated and characterized Spike-specific CD4 and CD8 T-cells after vaccination in non-responders KTR and compared such cellular responses with those from vaccinated immunocompetent subjects.

RESULTS

No KTR (15 female, 20 male) nor HD (12 female, 4 male) individual had a previous diagnosis of Covid-19. The median age of KTR was 57 years (interquartile range, 50.2 to 65.7) and 47.5 in HD group (interquartile range, 41 to 56) ($p < 0.005$, *t*-test). Serological status after vaccination were not systematically determined for HD individuals. Among the 35 seronegative KTR included, 16 had detectable Spike-specific T-cells (45.7%). This was significantly lower compared to HD group, where all tested individuals had detectable Spike-specific T-cells (100%) ($p < 0.001$, *Chi-square test*).

MATERIALS AND METHODS

All participants were recruited at University Hospital of Nancy (France). Thirty-five seronegative non-responders KTR vaccinated with 2 doses of Cominarty® were included. Sixteen healthy subjects (HD) vaccinated either with 2 doses of Cominarty® ("Pfiz") or 1 dose of Vaxzevria® followed by 1 dose of Cominarty® ("Oxf+Pfiz") were also evaluated. Spike-specific memory CD4 and CD8 T-cells were evaluated by a standard intracellular cytokine staining assay in flow cytometry 3 to 6 weeks after the second vaccination shot. Fresh PBMC were separated by density gradient and washed, 1×10^6 cells were resuspended in 200 μ L of AIM-V medium and stimulated overnight (14-16 hours) with peptide pools of overlapping 15-mer peptides spanning the Spike SARS-CoV-2 protein (divided in two pools of peptides: "SK1" and "SK2") (1.25 μ g/mL of each peptide). All samples were also co-stimulated with antibodies to CD28 and CD49d (1 μ g/mL, BD Biosciences). Brefeldin A (Sigma-Aldrich) was added to each well to block cytokine secretion. Unstimulated PBMCs (medium + 0.5% DMSO, vehicle of the peptide pools) were used as negative controls. As positive controls of global TH1-immunocompetence, PBMC were also stimulated in parallel with PMA (50 ng/mL, Sigma-Aldrich) and Ionomycin (500 ng/mL, Sigma-Aldrich). All peptide pools were from JPT Peptides (Berlin, Germany). After stimulation, cells were collected and washed before an incubation with BD Horizon™ Fixable Viability Stain 450 (FVS 450) and a cell surface antibody mix of anti-CD3 PerCP-Cy5.5, anti-CD4 FITC and anti-CD8 APC-H7. Cells were then fixed, washed and permeabilized by using the IntraStain® kit (Dako). Next, the cells were incubated with an intracellular antibody mix of anti-IL-2 PE, anti-TNF- α PE-Cy7 and anti-IFN- γ APC. All conjugated antibodies were from BD Biosciences. After 2 washes, cells were resuspended in PBS and immediately proceeded to flow cytometry data acquisition. Data were acquired using a BD FACSLyric™ @ flow cytometer (Becton Dickinson). SARS-CoV-2 -specific T-cells were identified using Kaluza® software v2.1 (Beckman Coulter) as by a specific gating strategy and a response was considered positive if greater than 0.01% of CD4+ or CD8+ T-cells were cytokine positive over background controls (Winstone *et al.*, Eur J Immunol. 2009). To quantify the polyfunctionality of SARS-CoV-2 specific T-cells, we calculated the Special Polyfunctionality index for each stimulation condition for CD4 and CD8 T-cells as described (Larsen *et al.*, PLoS One. 2012), using a higher ponderation for increasing polyfunctionality as determined by analysis correlating T-cell polyfunctionality and efficacy (Boyd *et al.*, PLoS One. 2015).

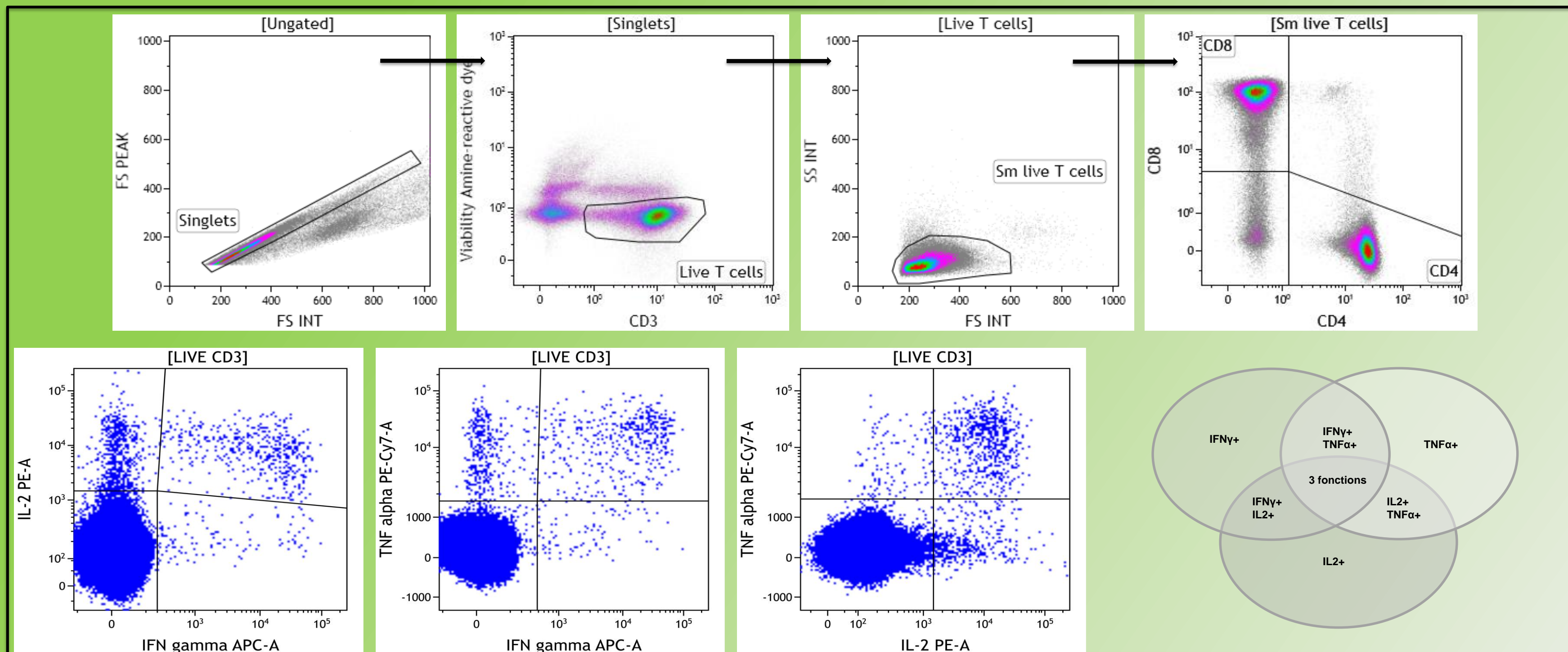


Figure 1. Gating strategy for identification of SK-1 and SK-2 memory specific T cells. Singlet cell population was identified by applying an FSC-Int vs. FSC-Peak gate. Next, live T-cells were identified by a gate on CD3+ and low viability Fixable Dye staining. Small viable T-cells were then identified as low SSC CD3+ cells and a CD4 vs CD8 gate was applied. A quadrant gate was set using the negative controls (not shown) for the baseline expression of each cytokine on CD4 and CD8 T-cells, defined as a percentage of IL-2+, TNF- α + or IFN- γ + cells. The same gating strategy was then used for SK1 and SK2 -stimulated cells. Mono, bi and trifunctional T cells were identified by Boolean gates using Kaluza® software. Seven populations can be then identified. Shown here are examples of cytokine-positive CD3 T-cells after SK1 stimulation and the resulting bifunctional responses.

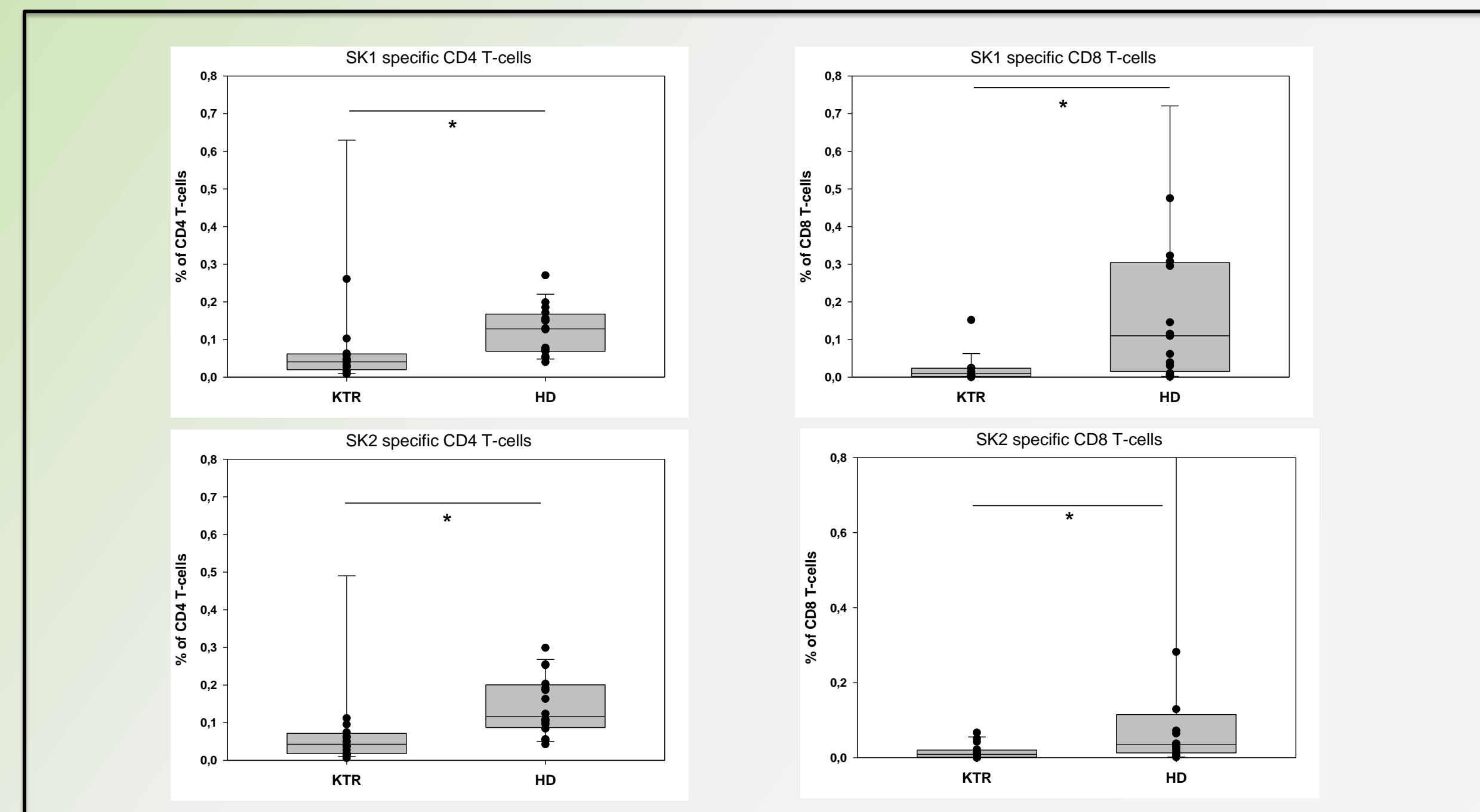


Figure 2. Global CD4 and CD8 Spike-specific T-cells. No significant differences were found between "Pfiz" and "Oxf+Pfiz" vaccination schedules in immunocompetent subjects. They were therefore pooled as HD group. KTR had significantly lower CD4 and CD8 T-cells specific to Spike-1 and Spike-2 peptide pools as compared to HD. The global response (adding all 7 subpopulations of cytokine-positive cells) is shown. Median and the 25th and 75th percentiles, whiskers represent the 10th and 90th percentiles. Black dots represent individual data. * $p < 0,05$

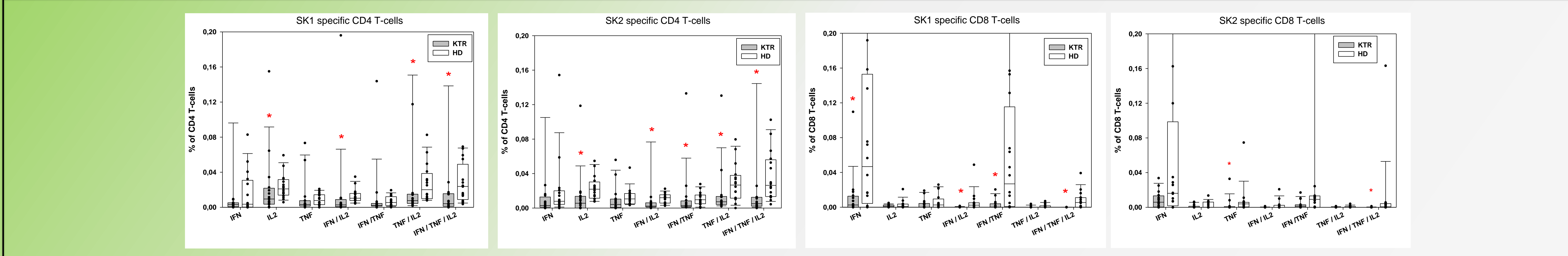


Figure 3. Proportions of mono and polyfunctional Spike-specific CD4 and CD8 T-cells. We looked for polyfunctional T cell responses as they are associated with a higher anti-viral efficacy. Overall, there was lower polyfunctional CD8+ T-cell responses in both groups. Compared to HD group, KTR had significantly lower proportions of trifunctional IL-2+, TNF- α + and IFN- γ + CD4 and CD8 T-cells. Bifunctional CD4 and CD8 T-cells were also lower in KTR, reaching statistical significance in most subpopulations. * $p < 0.05$

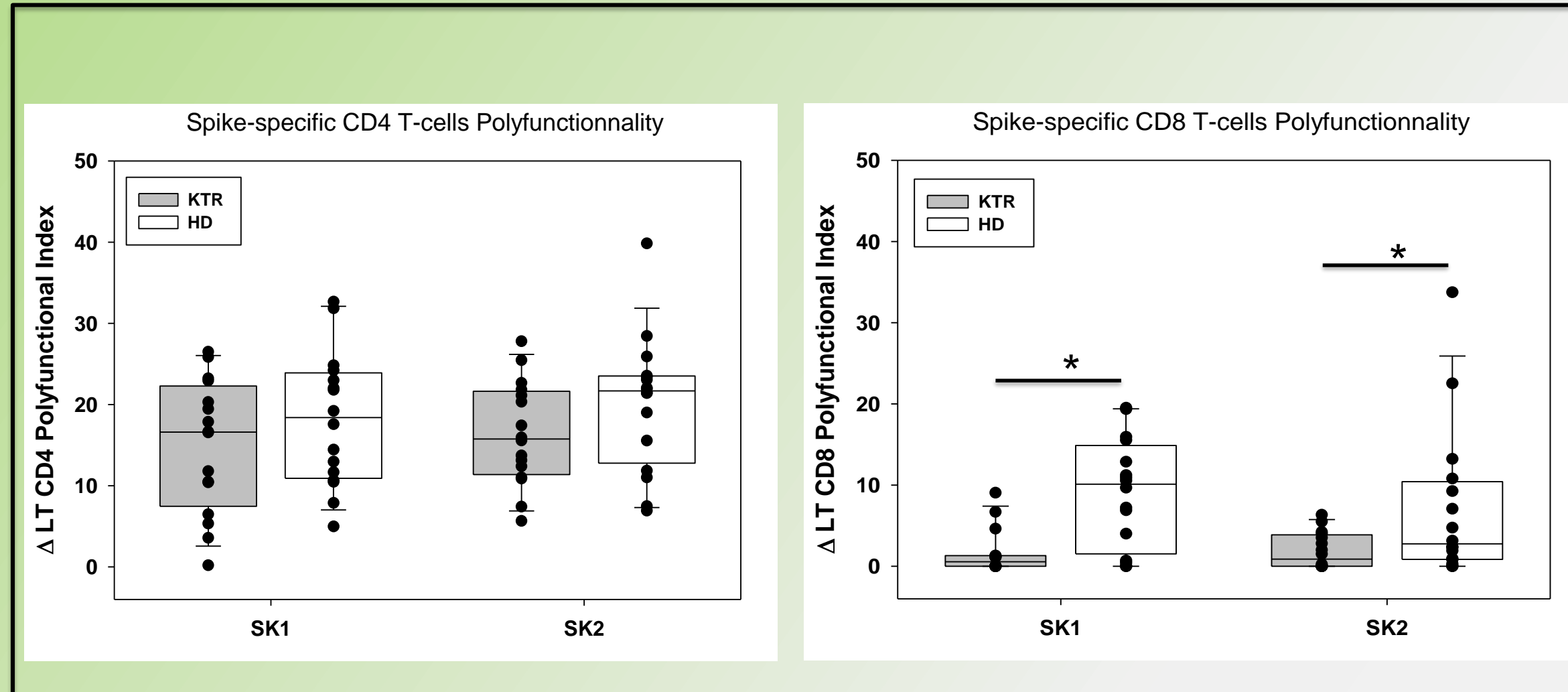


Figure 4. Quality of anti-Spike T-cells as evaluated by the Polyfunctionality index. Negative controls present a background production of cytokines (mostly monofunctional cells), we then subtracted the Polyfunctional index of negative controls from each specific antigenic stimulation to correct for this "background polyfunctionality" for each patient tested. The results are therefore expressed as Δ polyfunctional index. KTR Spike-specific CD8 T-cells had a significantly lower Polyfunctionality index compared to HD; * $p < 0.05$

CONCLUSIONS

The results of this ongoing work show that, despite a negative humoral response after a complete mRNA-based SARS-CoV-2 vaccination, an important proportion of KTR develop cellular responses after vaccination. However, Spike-specific CD4 and CD8 T-cells are present in lower numbers, notably the more efficient polyfunctional T-cells, when compared to healthy immunocompetent vaccinated individuals. Currently, there is no clear clinical correlate of disease protection defined for T-cell response magnitude after SARS-CoV-2 infection, but the assessment of cellular immunity might be useful before deciding the administration of prophylactic monoclonal antibodies in case of no humoral response to vaccination. Further studies are warranted to confirm these data, including different contexts of immunosuppression.