Lower T cell response against SARS-CoV-2 variants of concern after mRNA vaccine and risk of breakthrough infections in people living with HIV.

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Introduction

SARS-CoV-2 variant-of-concern (VOC) B.1.1.529 (Omicron) presents a surprisingly large number of mutations in its spike protein escaping from antibody neutralization. Thus, it is important to determine how well T-cell response performs against different variants including Omicron in people living with HIV (PLH) following COVID-19 vaccination, and the impact on new infections during follow up.

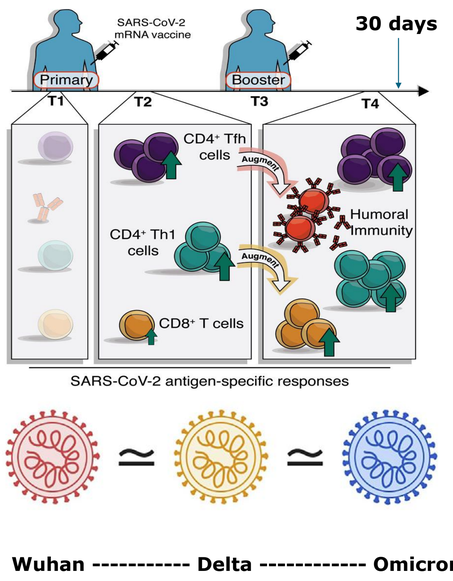
Objective

To evaluate, after two doses of mRNA vaccination against SARS-CoV-2, the CD4+ and CD8+ T cell response to the different variants: ancestral (Wuhan), Delta, and Omicron variants in PLH.

Methods

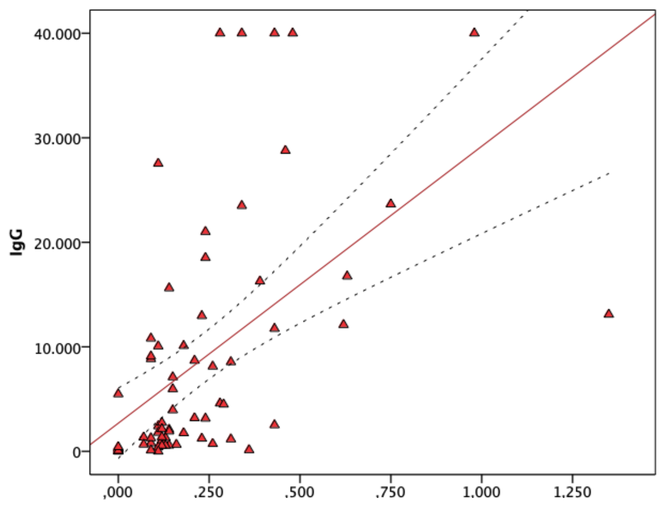
Pilot study of PLH who underwent blood tests for humoral and cellular response after 30 days of the two-doses vaccination schedule with an mRNA vaccine against SARS-CoV-2. Humoral (anti-S IgG, CLIA, Abbott, USA; limit of positivity 7 BAU/ml) and IFN-γ producing T cell responses to spike peptides of the ancestral virus, and delta and omicron variants were performed.

SARS-CoV-2-specific CD4+ and CD8+ T-cells were measured using *in vitro* stimulation with SARS-CoV-2 overlapping peptide pools spanning the entire spike protein sequences of the Wuhan reference strain (wildtype), delta, and omicron variants, followed by quantitation of CD4+ and CD8+ T-cell specific interferon (IFN)-γ in live cell flow cytometry, using peripheral blood mononuclear cell (PBMC) samples from all subjects. It was considered significantly reactive if the proportion of positive cells in stimulated wells was at least 2-fold higher in comparison with the negative control wells (unstimulated).
In detail, EDTA-blood samples were collected from all individuals. After centrifugation at 200g for 10 min, plasma fraction was collected and again centrifuged at 1200g for 15 min, aliquoted and stored at -80ºC. The cellular fraction was diluted with phosphate-buffered saline (PBS) and subjected to Ficoll density gradient centrifugation at 500g for 20 min. PBMCs were washed and frozen in fetal bovine serum (FBS) with 8% dimethyl sulfoxide (DMSO, Sigma, USA) in liquid nitrogen. PBMCs were thawed and plated in 96-well flat-bottom plates at 10^6 cells/well in RPMI-1640 culture medium (Gibco, USA) supplemented with 10% human serum (AB serum, Sigma), 100 IU of penicillin/streptomycin/mL (Gibco, USA), 2 mM L-glutamine, and after 24 hours cells were stimulated in five different conditions in the presence of 1 µg/ml purified anti-CD28 antibody (Miltenyi, Germany). Three wells were stimulated with each of the SARS-CoV-2 peptide pools S of Wuhan strain (wild type), delta and omicron variant at a concentration of 1 µg/ml. Each peptide pool was composed of 15-mer sequences with 11 amino acids overlap, covering the immunodominant sequence domains of the surface glycoprotein S (PepTivator SARS-CoV-2 Prot S, Miltenyi-Biotec, Cologne, Germany). In addition, one well was cultured with complete medium as an unstimulated, and other well was stimulated adding 1.5 mg SEB (staphylococcal enterotoxin B, Sigma, Germany) as the positive control. An unresponsive sample to SEB would be excluded from the analysis.
Stimulated PBMCs were incubated for two hours before adding brefeldin A (Rapid Cytokine Inspector CD4/CD8 T cell kit, Miltenyi, Germany) into the medium to stop cytokine release and kept in culture for other 14 hours. After incubation, staining of the cells was carried out with the following fluorochromes-conjugated antibodies using Rapid Cytokine Inspector CD4/CD8 T cell kit (Miltenyi, Cologne, Germany): CD3-VioBlue, CD4-APC, CD8-FITC, CD14-PerCP, CD20-PerCP, IFN-γ-PE, and FcR blocking reagent. To exclude dead cells, viability 405/520 fixable dye staining (Milteny, Cologne, Germany) was added for the last 10 min of incubation. Fixation and permeabilization were performed according to the manufacturer´s protocol. Samples were measured and analyzed by flow cytometry on a MACSQuant Analyzer 10 using MACSQuantify software. Cells were analyzed and gated with the following strategy. Single (FSC-A/FSC-H dot plot) and live cells were first selected. Cell debris, monocytes, and B cells were excluded from the analysis with CD14- and CD20-PerCP antibodies. Then, lymphocytes were selected with a FSC-A/SSC-A dot plot, and CD3 T cells were gated. IFN-γ expression was finally analyzed for CD4+ and CD8+ T cells, and expressed by percentage. The cytokine expression was considered significant if there was at least 2-fold increase in stimulated wells in in comparison with unstimulated control well.



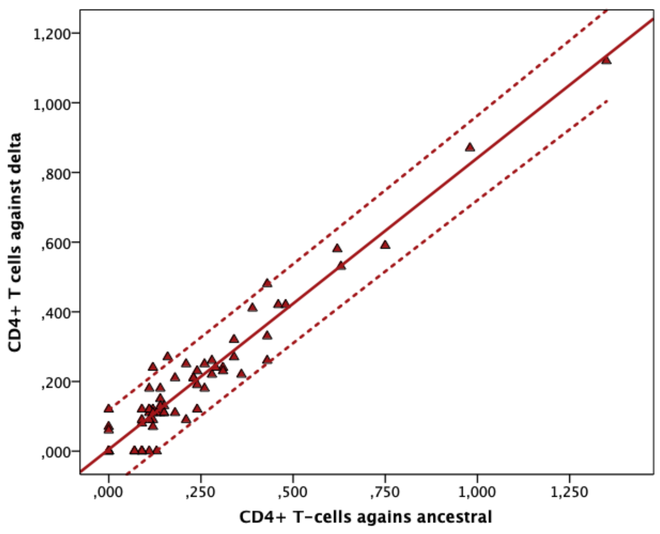
Wuhan ---------- Delta ---------- Omicron

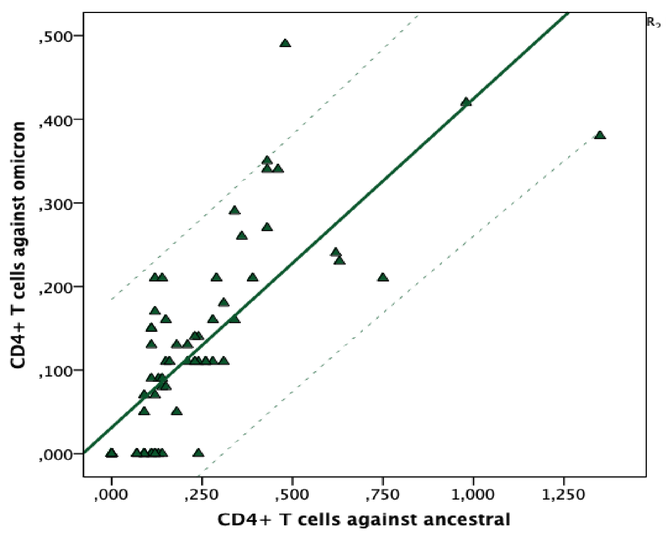
Correlation between humoral and CD4 T cell response to ancestral virus



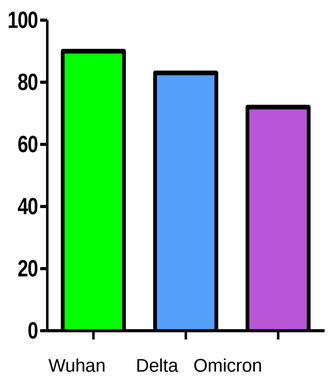
rho=0.657; p<0.01 between IgG and CD4+ T cells

Correlation between CD4 T cell response to ancestral virus, Delta variant and Omicron variant

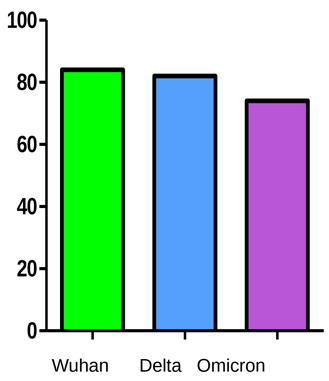




p<0,001 | *p=0,081* | **p=0,023**

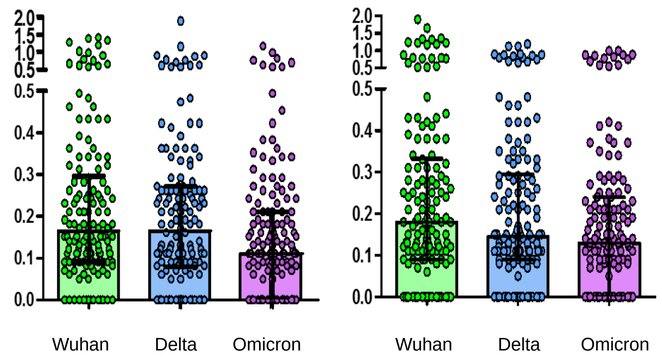


p=0,116 | p=0,526 | **p=0,028**



p<0,001 | p=0,004 | **p=0,007**

p=0,012 | p=0,007 | p=0,163



Conclusion

T-cell responses against Delta and Omicron spike peptides, although preserved in nearly two thirds of PLH, were significantly lower than to the original strain after two doses of mRNA vaccine.

Importantly, this lower response was associated with breakthrough infections during follow-up.

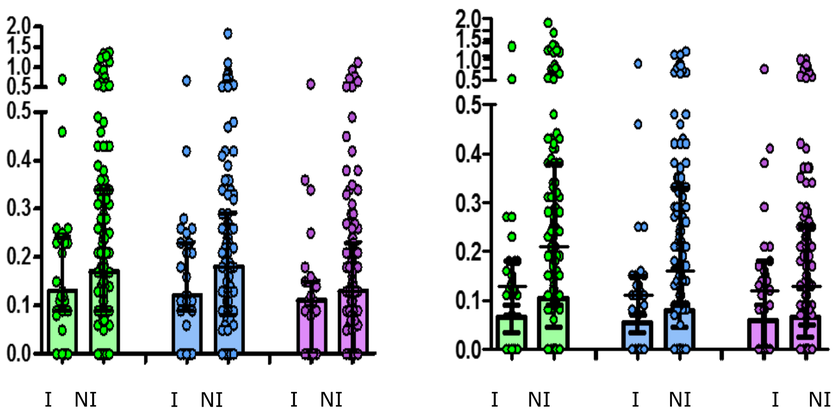
Baseline characteristics (n=142)

Median age (years, range)	53 (24-78)
Sex male	118 (83)
Risk practice MSM	107 (75)
BMI (kg/m2) Obesity	25.2 (23-27) 10 (7)
Comorbidities (1 or more)	58 (41)
Time from HIV diagnosis (months, median, IQR)	170 (75-374)
Nadir CD4+ (cells/mm^3), median, IQR	273 (48-350)
Previous AIDS	37 (26)
Current CD4+ (cells/mmc), median, IQR	659 (552-882)
Time from 2nd dose of vaccine (days, median, IQR)	53 (46-85)
CD4/CD8 ratio nadir, median IQR CD4/CD8 ratio current, median, IQR	0.15 (0.1-0.38) 0.88 (0.56-1.2)

Only 70% of PLH had T cell response to the three different variants

CD4+ | CD8+

p=0,203 | p=0,228 | p=0,179 | **p=0,020** | **p=0,034** | p=0,293



I, infected
NI, not infected

The magnitude of CD4+ and CD8+ T-cell responses were significantly lower to Delta in proportion (83% and 82% against Delta variant, 72% and 74% against Omicron variant, respectively), and in magnitude (3% and -20% for Delta, -33% and -28% for Omicron variant).

A total of **29 (17%) breakthrough infections were observed during a median follow-up of 351 days**, associated with a lower level of specific antibodies (890.8 vs 1559.7 BAUs; p=0.027), and with a lower magnitude of CD4+ and CD8+ T cell response to the different variants (statistically significant for CD8+ T-cell response against ancestral and Delta variants).

The only factor associated with a blunted T cell response was a lower increase in CD4+ count or CD4/CD8 ratio, suggesting that in some PLH the presence of incomplete immune reconstitution, despite effective virological suppression on antiretroviral therapy, may generate suboptimal T cell responses

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