Monitoring of vaccine-induced SARS-CoV-2-specific cellular immune responses

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Introduction

The global outbreak of the COVID-19 pandemic has emphasized the importance of immune monitoring technologies to reveal the dynamics of the immunological response in naturally acquired immunity, vaccination, and hybrid immunity. Immune monitoring technologies can help provide guidance to clinicians and health authorities about the type, magnitude, and duration of cellular immunity and thus improve the decision-making about future vaccine programs and roll out of booster doses.

Our aim was to develop SARS-CoV-2-specific immune monitoring assays based on the Dextramer® technology to detect and characterize virus-specific T-cell responses. The applicability of the Dextramer® assay for immune monitoring in SARS-CoV-2 was demonstrated by a longitudinal study on a small cohort vaccinated two or three times in total.

Detection of the longitudinal T-cell responses in SARS-CoV-2 by Dextramer® assay

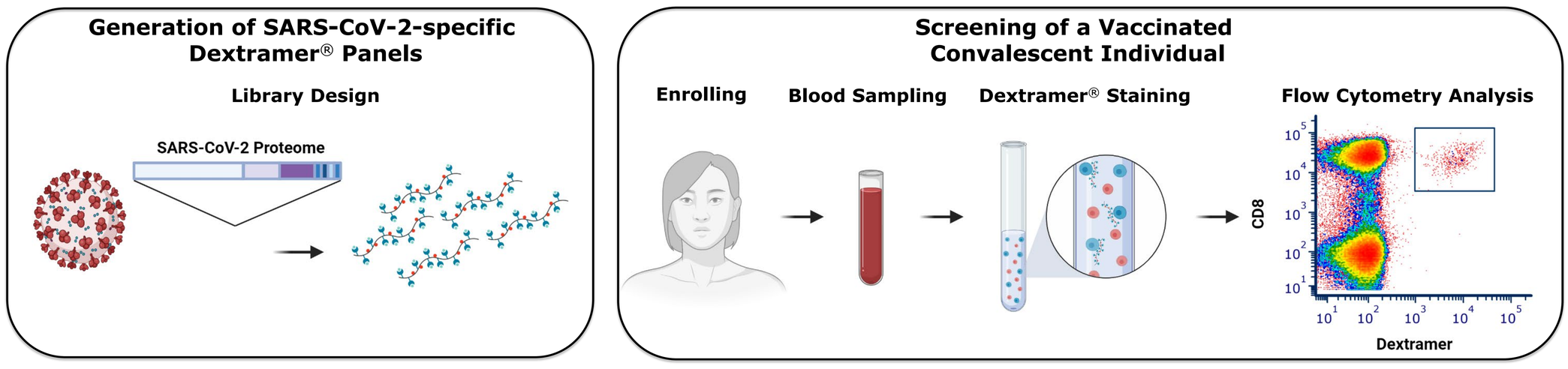

Figure 1. Schematic overview of the experimental workflow for Dextramer® staining of the CD8⁺ T-cell response to SARS-CoV-2. To detect SARS-CoV-2-specific CD8⁺ T cells in peripheral blood mononuclear cells (PBMCs), a SARS-CoV-2 MHC Dextramer® assay was developed. The assay was designed to cover seven of the most common class I human leukocyte antigen (HLA) alleles including A*01:01, A*02:01, A*03:01, A*11:01, A*24:02, B*07:02 and B*35:01 complexed to epitopes from the Spike protein of SARS-CoV-2. To evaluate the clinical performance of the assay, consecutive PBMC samples were collected from 24 individuals before 1st vaccination and after 2nd vaccination. For nine individuals additional samples were collected just before 3rd and after 3rd vaccination. The PBMCs were evaluated in the Dextramer® assay to determine the frequency of SARS-CoV-2-reactive CD8⁺ T cells. Briefly, PBMCs were stained in a tube containing allele-matched Dextramer® reagents displaying epitopes from Spike (PE labelled) and Dextramer® reagents displaying nonsense epitopes (APC labelled) as negative control. In a second tube, cells were labelled with a universal Dextramer® reagent (PE labelled) as positive control. Samples were analyzed using flow cytometry. Images were created with BioRender.com.

Dextramer® assay reveals changes in magnitude and kinetics of SARS-CoV-2-specific CD8⁺ T-cell immunity

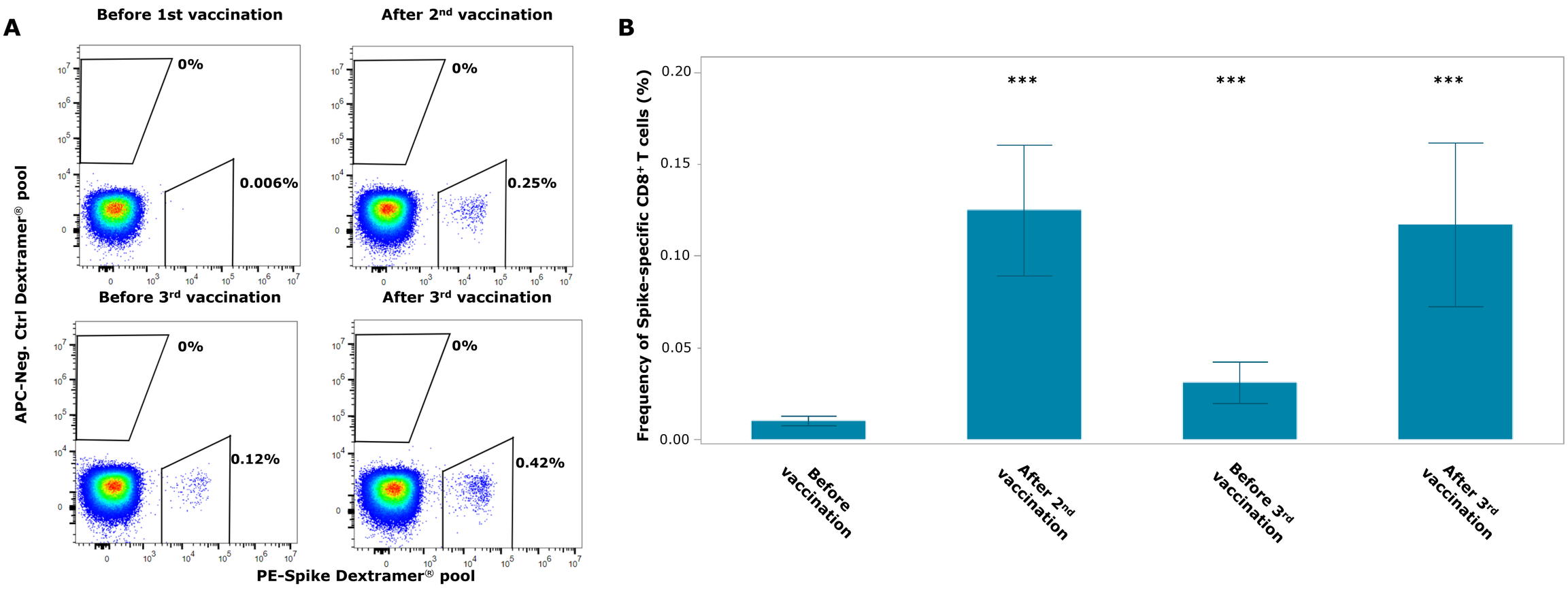

Figure 2. The Dextramer® assay detects SARS-CoV-2-specific CD8⁺ T-cell responses displaying different magnitude and kinetics. (A) Representative flow cytometry plots showing SARS-CoV-2-specific CD8⁺ T cells reactive with Spike epitopes detected in PBMCs from a vaccinated individual. The frequencies are here defined as % frequency of SARS-CoV-2-specific CD8⁺ T cells of total count of CD8⁺ T cells. A CD8⁺ T-cell response was detected for all time points after vaccinations. There was no detectable response against Spike in the baseline sample. **(B)** Bar plot summarizing the change in the frequencies of Spike-specific CD8⁺ T cells across time points. Data showed first an increase in the levels of Spike-specific CD8⁺ T cells after 2nd vaccination followed by a decreased level just before 3rd vaccination and then followed by an increased level after the 3rd booster vaccination. The Wilcoxon signed-rank test was used to compare paired samples before and after vaccination(*** $p \leq 0.001$).

CD8⁺ and CD4⁺ T-cell epitopes are highly conserved across SARS-CoV-2 reference strain, Delta and Omicron Variants

Protein	T cells	Reference	Delta	Omicron
Spike	CD8⁺	17/17 (100%)	15/17 (88%)	14/17 (82%)
Non-Spike	CD8⁺	17/17 (100%)	16/17 (94%)	17/17 (100%)
Spike	CD4⁺	17/17 (100%)	15/17 (88%)	13/17 (76%)
Non-Spike	CD4⁺	12/12 (100%)	12/12 (100%)	12/12 (100%)

Table 1. Blast analysis of T-cell epitopes. T-cell epitopes used in the assay were blasted against Spike and Non-Spike proteins of the SARS-CoV-2 reference strain (NC_045512), Delta (B.1.617.2) and Omicron (B.1.1.529) variants to obtain the degree of eptiope conservation between SARS-CoV-2 strains. The blasts showed a high degree of conservation of CD8⁺ T-cell epitopes from Spike as well as Non-Spike protein. A selection of CD4⁺ T-cell epitopes were blasted, too, and showed a high degree of conservation across SARS-CoV-2 strains. All the analyzed T-cell epitopes were identified by reviewing published literature and verified by Immudex using inhouse assays.

Conclusion

We have developed a broad HLA class I Dextramer® assay that can monitor the dynamics of CD8⁺ T-cell responses against Spike protein of SARS-CoV-2. The SARS-CoV-2-specific Dextramer® assay was evaluated in a longitudinal cohort study to assess the time-dependent changes in the levels of SARS-CoV-2-specific T cells using samples from time-points of before 1st vaccination, after 2nd vaccination, and before and after 3rd vaccination. Spike-specific CD8⁺ T-cell responses demonstrated different patterns of magnitude and kinetics. These results demonstrate that the SARS-CoV-2 Dextramer® assay can **(1)** assess long-term immunity upon vaccination, **(2)** support decision-making about the need for booster doses if the immunity declines and **(3)** guide future vaccine development.

Additionally, a blast analysis of Spike epitopes revealed that the CD8⁺ T-cell epitopes used in the assay are highly conserved in Delta and Omicron strains, and thus the SARS-CoV-2 Dextramer® assay is applicable for studies not only of Spike-based vaccines, but also of infection with ”reference strain”, Delta and Omicron strains. Similarly, blast analysis of Non-Spike CD8⁺ T-cell epitopes and CD4⁺ T-cell epitopes to Spike and Non-Spike proteins highlighted the conservation of CD8⁺ and CD4⁺ T-cell epitopes across SARS-CoV-2 strains. Currently, the SARS-CoV-2 Dextramer® assay is being further evaluated to include CD8⁺ and CD4⁺ T-cell epitopes against Spike and Non-Spike proteins for broader immune response monitoring after vaccination or viral infection.