Monitoring of Naturally Acquired and Vaccine-Induced SARS-CoV-2-Specific CD8⁺ T-Cell Responses

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Introduction

The T cell immune response against SARS-CoV-2 is a determining factor in the clinical course of natural infection and the efficacy of vaccines. This has emphasized the importance of immune monitoring technologies that can help guide clinicians and health authorities about the type, magnitude, and duration of cellular immunity and thus improve the decision-making about current and future vaccine programs [1, 2].

We aimed to develop SARS-CoV-2-specific immune monitoring assays based on the Dextramer® technology to detect and characterize virus-specific CD8⁺ T-cell responses. The applicability of the Dextramer® assays for immune monitoring in SARS-CoV-2 was demonstrated in a small cohort of naïve and convalescent individuals vaccinated towards SARS-CoV-2.

Rapid detection of T-cell responses in SARS-CoV-2 by Dextramer® assays

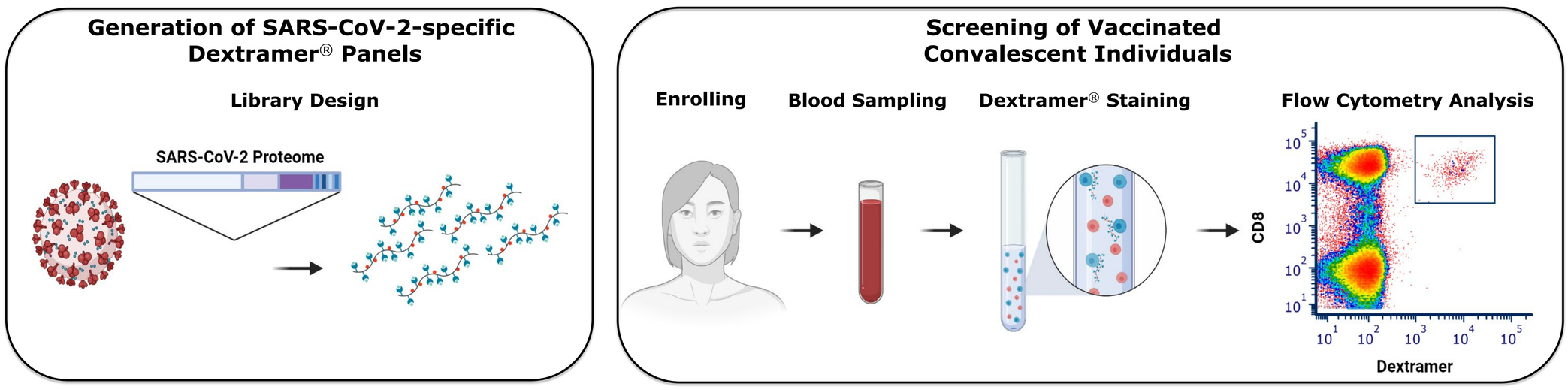

Figure 1. Overview of the experimental workflow to Dextramer® staining of the CD8⁺ T-cell responses to SARS-CoV-2. To detect SARS-CoV-2-specific CD8⁺ T cells in peripheral blood mononuclear cells (PBMCs), two SARS-CoV-2 MHC Dextramer® assays targeting Spike and Non-Spike-specific T cells, respectively, were developed. The assays covered 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 Spike, Nucleocapsid, ORF1a, ORF1ab and ORF3a proteins of SARS-CoV-2. To evaluate the applicability of the assays, PBMCs were collected from 24 naïve and 11 convalescent individuals before first vaccination and after dual vaccination towards SARS-CoV-2. The PBMCs were evaluated in the Dextramer® assays to determine the frequency of SARS-CoV-2-reactive CD8⁺ T cells. Briefly, the PBMCs were stained in a tube with allele-matched Dextramer® reagents displaying epitopes from Spike (PE labeled) (Spike Dextramer® assay), or in a tube with Spike (PE labelled) and Non-Spike (APC labeled) Dextramer® reagents (Non-Spike Dextramer® assay). Positive and negative control Dextramer® reagents were included as assay references. Upon Dextramer® staining, the PBMCs were subjected to an antibody cocktail towards CD8, CD3, CD45RA, CCR7, CD27 and HLA-DR. Samples were analyzed using flow cytometry.

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

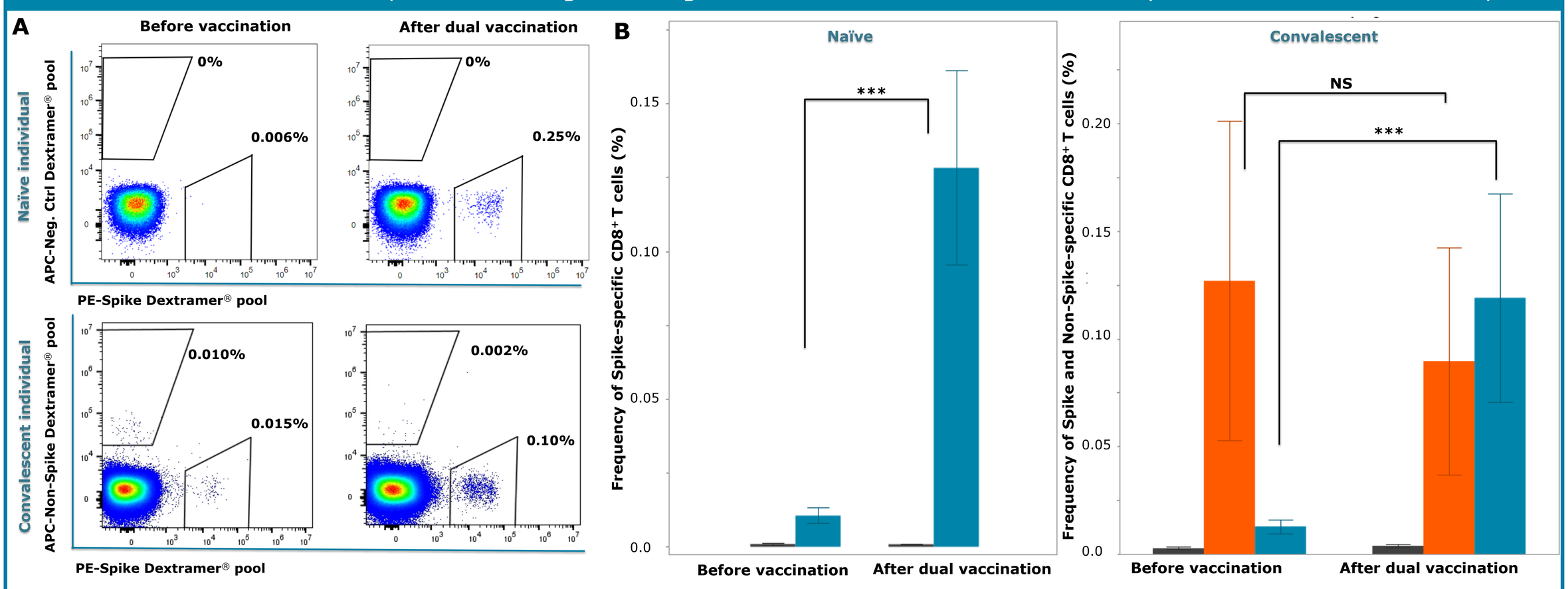

Figure 2. The Dextramer® assays monitor SARS-CoV-2-specific CD8⁺ T-cell responses in naïve and convalescent individuals before and after dual vaccination with a Spike-based vaccine. (A) Representative flow cytometry plots showing SARS-CoV-2-specific CD8⁺ T cells reactive with Spike and Non-Spike epitopes detected in PBMCs from a naïve and a convalescent individual, respectively. The frequencies are defined as the % frequency of SARS-CoV-2-specific CD8⁺ T cells of the total count of CD8⁺ T cells. **(B)** Bar plots summarizing the change in the frequencies of SARS-CoV-2-specific CD8⁺ T cells before and after dual vaccination for all subjects analyzed. A Spike-specific T-cell response was detected in 22/24 naïve individuals upon dual vaccination. The convalescent individuals showed no or very weak Spike-specific T-cell responses before vaccination with levels comparable to naïve individuals. A Non-Spike T-cell response was detectable at both timepoints in 10/11 convalescent individuals. The Wilcoxon signed-rank test was used to compare paired samples before and after vaccination (*** $p \leq 0.001$, NS not significant). Orange, Non-Spike-specific CD8+ T cells, Blue, Spike-specific CD8+ T cells, Dark grey, negative control.

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 analysis 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 published literature review and verified in in-house assays by Immudex.

Conclusion

- Two HLA class I Dextramer® assays with broad Spike and Non-Spike epitope coverage were successfully developed for the monitoring of CD8⁺ T-cell responses against SARS-CoV-2 upon infection and vaccination.
- The developed SARS-CoV-2 Dextramer® assays successfully assessed the changes in the levels of SARS-CoV-2-specific T cells before vaccination and after dual vaccination in naïve and convalescent individuals of a small patient cohort.
- A BLAST analysis of Spike and Non-Spike epitopes revealed that the CD8⁺ T-cell epitopes used in the assays 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.
- The SARS-CoV-2 Dextramer® assays are efficient tools for **(1)** monitoring of T cell immunity upon vaccination and infection, **(2)** supporting decision-making about the need for booster doses if the immunity declines and **(3)** guiding future vaccine development.

References
[1] Moss, P. The T cell immune response against SARS-CoV-2, Nature Immunology 2022
[2] Jung et al. SARS-CoV-2-specific T cell memory is sustained in COVID-19 convalescent patients for 10 months with successful development of stem cell-like memory T cells, Nature 2021

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