Simultaneous detection and characterization of antigen-specific B cells and CD4+ and CD8+ T cell responses upon natural infection and vaccination

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Introduction

Understanding the antigen-specific B and T cell responses is key for development of vaccines and targeted therapies, encompassing various stages from target discovery to monitoring the treatment efficacy to patient stratification. The Dextramer[®] and Klickmer[®] reagents allow simultaneous detection of low-frequency ag-specific B and T cells in the same workflow. For a deeper investigation, the dCODE Dextramer[®] and dCODE Klickmer[®] reagents can be used in combination with single-cell RNA sequencing, which provides a deep dive into the ag-specific B and T cells at the individual cell level giving access to BCR/TCR sequences for specific targets.

Here we demonstrate two workflows in a SARS-CoV-2 model system for simultaneous detection of ag-specific B and T cells within the same sample using (1) Dextramer[®] and Klickmer[®] reagents in combination with flow cytometry or (2) dCODE Dextramer[®] and dCODE Klickmer[®] reagents in combination with the 10x Single-Cell Analysis System.

(1c)

Workflows for simultaneous investigation of antigen-specific B and T cells in blood samples

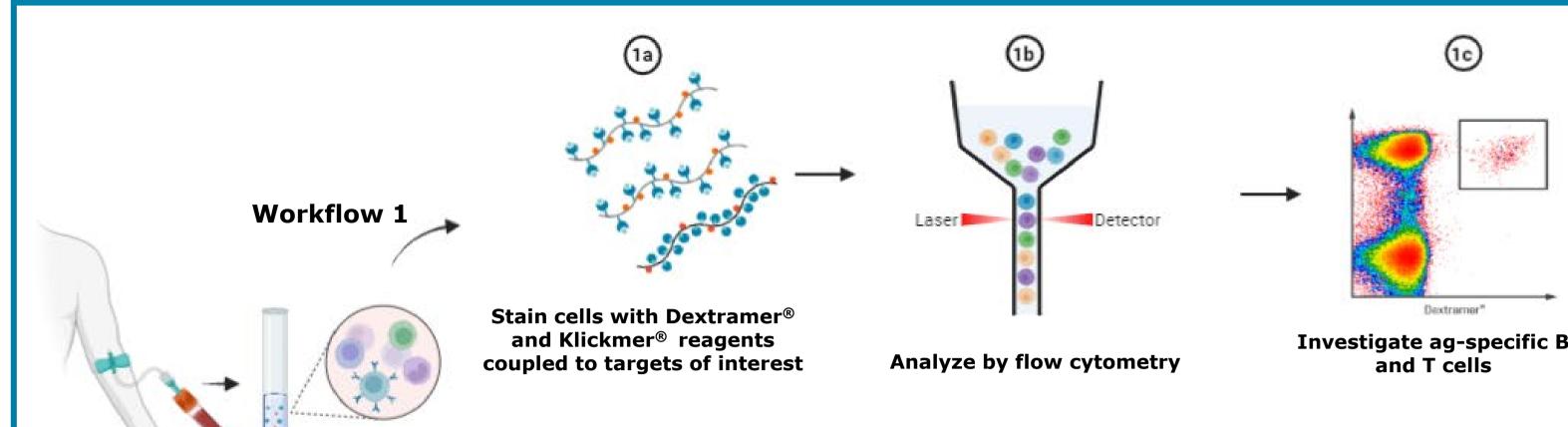
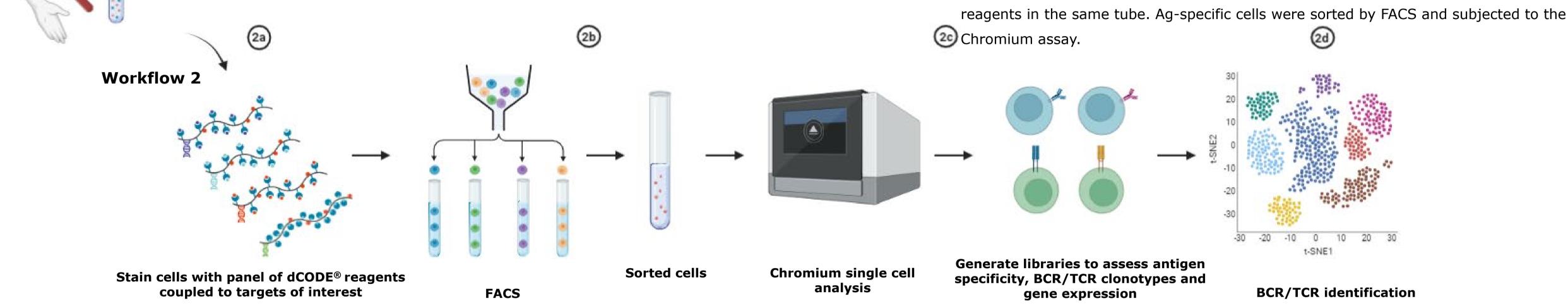
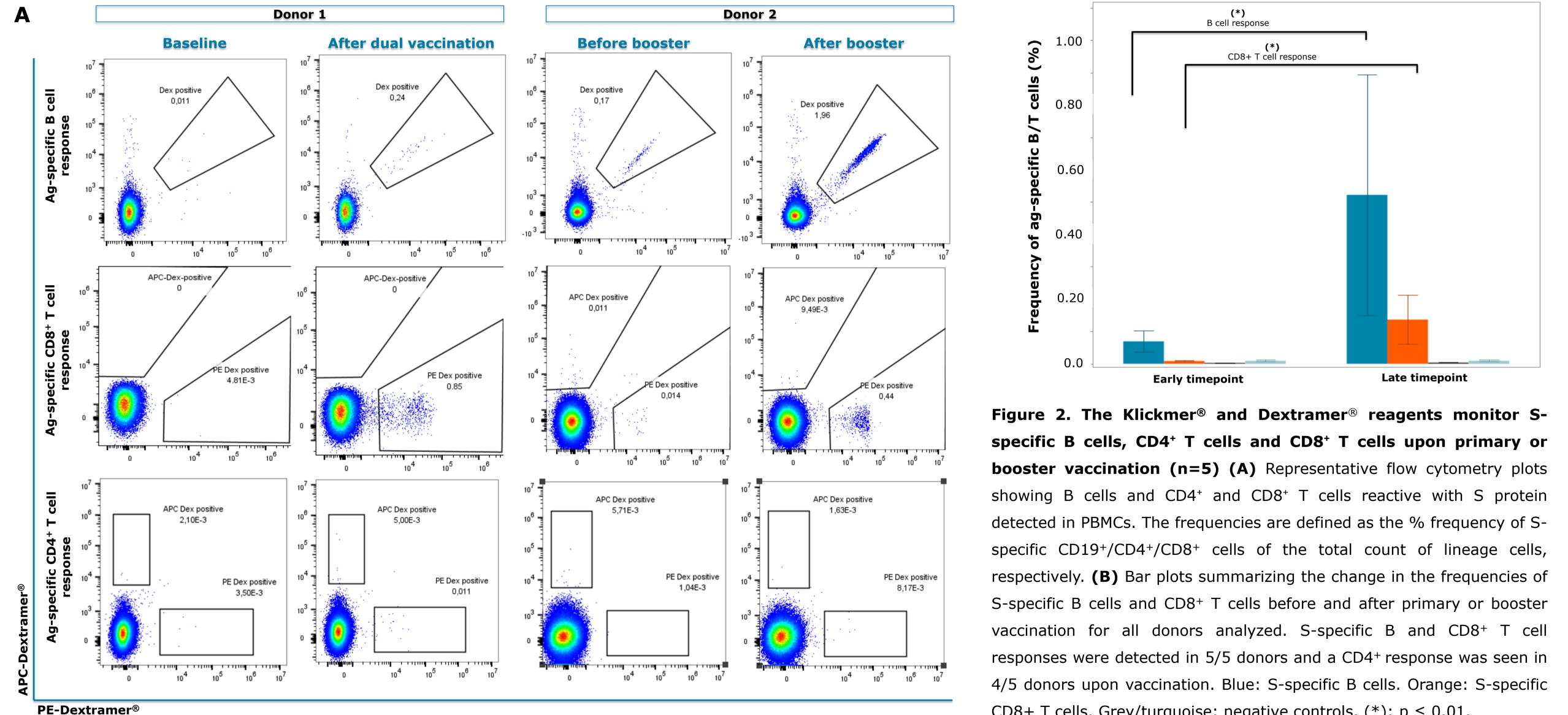


Figure 1. Overview of workflows. Workflow 1. To detect Spike (S)-specific B and T cells, we used MHC Dextramer[®] reagents targeting S-specific CD8⁺ and CD4⁺ T cells and a Klickmer[®] reagent coupled to full-length S to target B cells. PBMCs from each of 5 donors collected before and after vaccination towards SARS-CoV-2 were divided into three tubes and stained with CD4+, CD8+ or B cell Dextramer[®] and Klickmer[®] reagents. Control reagents were included as references. Upon Dextramer[®] staining, the PBMCs were subjected to an antibody pool towards CD8, CD4, CD3, CD14, and CD19. Samples were analyzed by flow cytometry. Workflow 2. To investigate the BCR/TCR profiles, two PBMC samples from a convalescent donor were stained with dCODE Dextramer[®] and dCODE Klickmer[®] reagents (n=21) targeting SARS-CoV-2specific (S, Nucleocapsid, ORF1ab) CD8⁺ T cells, S-specific B cells and control



Dextramer[®] and Klickmer[®] reagents reveal changes in magnitude and kinetics of antigen-specific B and T cells upon vaccination



CD8+ T cells. Grey/turquoise: negative controls. (*): $p \le 0.01$.

dCODE Dextramer[®] and dCODE Klickmer[®] reagents identify TCR and BCR clonotypes

J gene

TRBJ2-7

TRAJ42

TRBJ2-1

TRAJ49

TRBJ1-2

TRAJ41

IGHJ4

IGLJ2

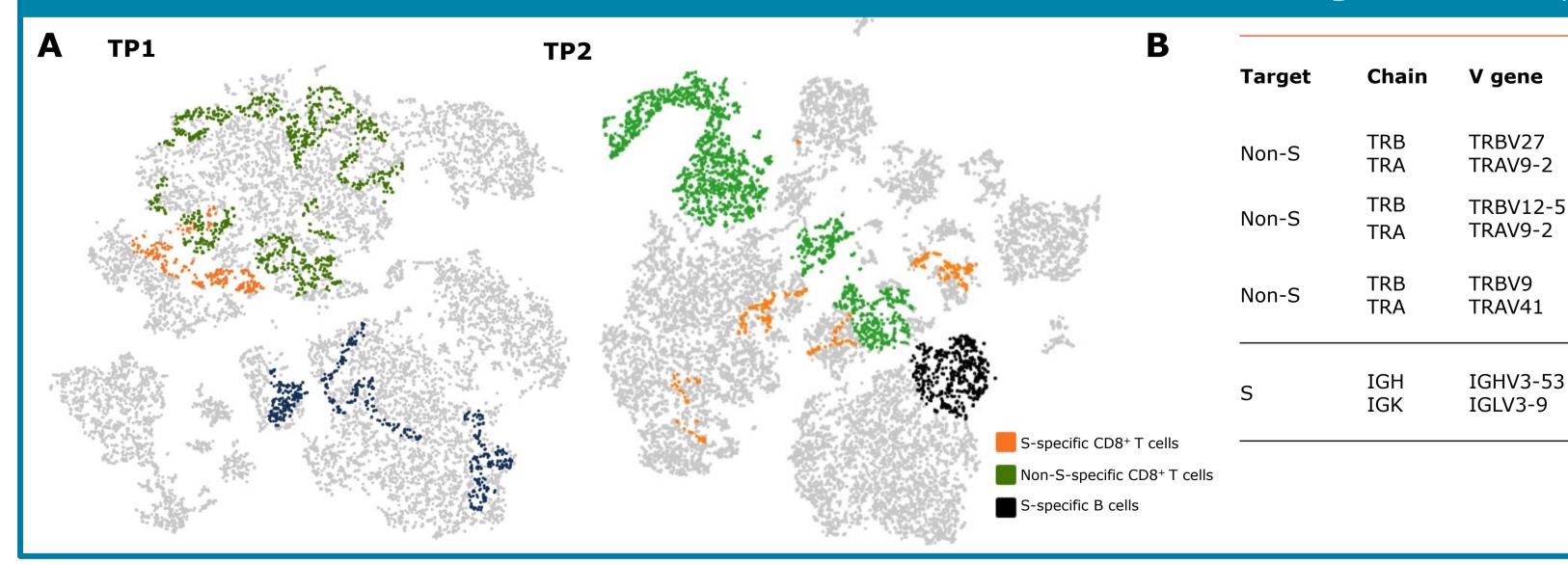


Figure 3. Dynamics of ag-specific B and T cells after a SARS-CoV-2 infection. (A) SARS-CoV-2 (S and Non-S) CD8⁺ T cell responses and S-specific B cell responses in donor samples collected immediately after (TP1) and six weeks after (TP2) a second SARS-CoV-2 infection. A total of 3408 (TP1) and 5680 (TP2) T cells and 4686 (TP1) and 4765 (TP2) B cells were called in the VDJ pipeline with a high frequency of productive V-J spanning pairs (> 78%). (B) List of TCR and BCR clonotypes. Representative data showing the persistence of some Non-S-specific CD8+ T TCR clonotypes and S-specific BCR clonotypes, cell respectively, at TP1 and TP2.

Conclusion

two workflows for the simultaneous detection and characterization of ag-specific B and T cells in blood samples. demonstrated We have

(1) Workflow 1 combines Dextramer[®] and Klickmer[®] reagents with flow cytometry to detect and characterize ag-specific B cells, CD4⁺ and CD8⁺ T cells. The workflow

was demonstrated using SARS-CoV-2 as a model system to detect changes ag-specific B and T cells upon vaccination.

(2) Workflow 2 combines dCODE Dextramer[®] and dCODE Klickmer[®] with single-cell RNA seq to enable the examination of T and B cells in the same sample at the

individual cell level and facilitates the evaluation of specific target BCR/TCR clonotypes. We are currently exploring data in greater depth.



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