Rapid Assay for Detection of SARS-CoV-2 Spike-Specific B Cells Upon Vaccination

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Introduction

The monitoring of antigen-specific B and T cells upon natural infection with SARS-CoV-2 or vaccination is essential to assess the durability, magnitude, and kinetics of the generated immune responses. Longitudinal monitoring of antigen-specific B and T cells is challenging due to the low frequency of these cells in the blood, and requires reliable technologies. Therefore, we developed an assay based on Immudex's proprietary Klickmer® technology to detect and characterize SARS-CoV-2 Spike-specific B cells. Here, we demonstrate the performance of this assay by applying it to detect and characterize Spike-reactive B cell responses in blood from four individuals vaccinated against SARS-CoV-2, and compare with samples collected before vaccination.

Klickmer® technology is a plug-and-play approach consisting of a dextran backbone with an optimized number of biotin-acceptor sites and fluorophore molecules, allowing the attachment of any mono-biotinylated antigen. Hence, the assay can easily be adapted to detect antigen-specific B cells with any specificity. Klickmer® enables the direct detection of rare or low-affinity antigen-specific B cells, without any need for upstream stimulation, enrichment or expansion, thus saving time and effort. Moreover, the DNA barcode labeled Klickmer® technology is the only commercial product for single-cell analysis of antigen-specific B cells.

Spike-Klickmer® Assay Design

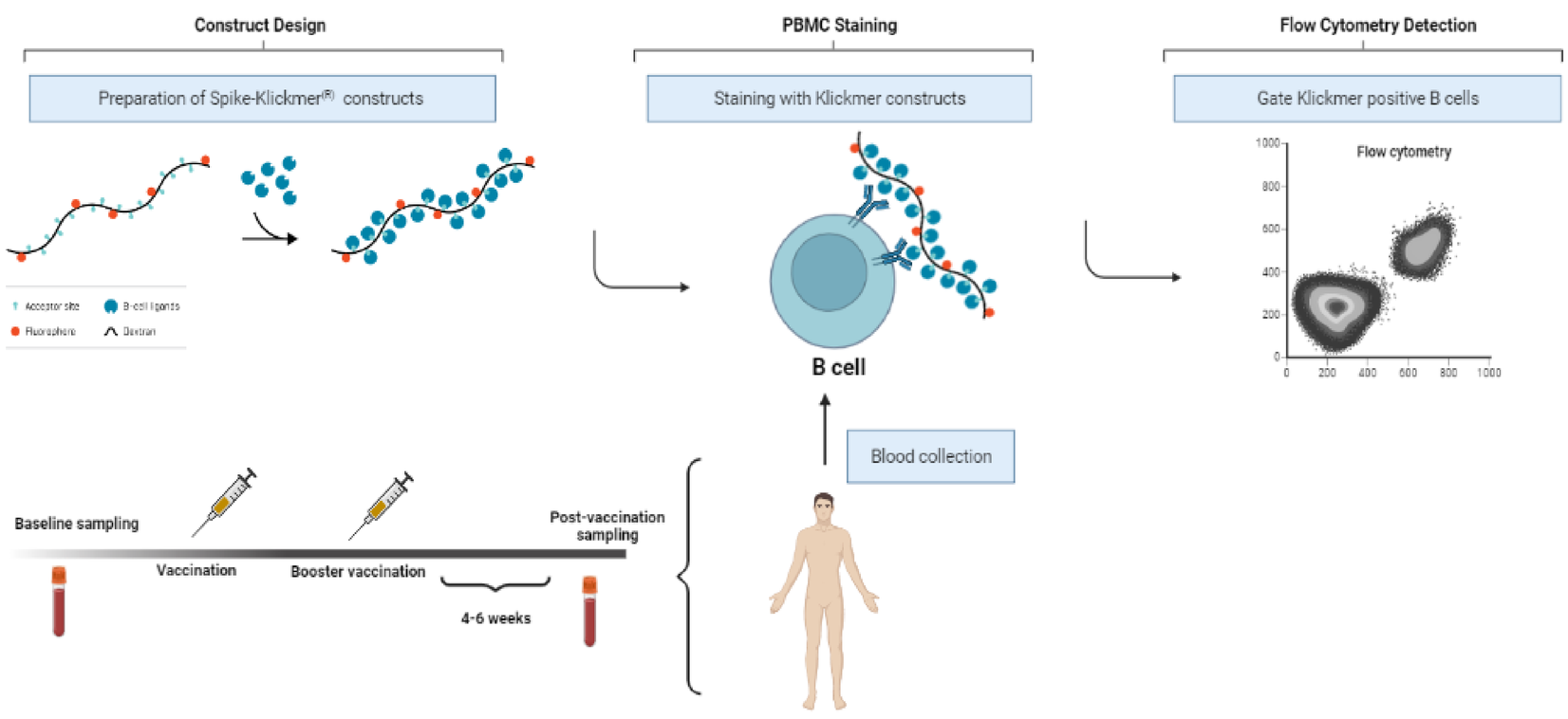

Figure 1. Assay Design A Spike-Klickmer® assay was developed to monitor Spike-reactive B cells upon vaccination. Spike protein of SARS-CoV-2 was coupled to Klickmer® reagents with two distinct fluorochromes, PE and APC, respectively. PBMCs were prepared from blood samples collected from healthy subjects (n=4) before and after SARS-CoV-2 vaccination. For a single donor, an additional sample was collected after a breakthrough infection. PBMC samples were stained using the two Spike-Klickmer® constructs with PE and APC in the same staining tube to detect Spike-reactive B cells. A universal negative control Dextramer® was included as reference. Upon staining with Spike-Klickmer® assay, the PBMCs were subjected to an antibody cocktail with antibodies to CD3, CD14, CD19, CD27, IgM, IgD, CD38 and CD138. Samples were analyzed by flow cytometry. Images were created with BioRender.com.

Detection and Characterization of Spike-Specific B Cells in Vaccinated Individuals

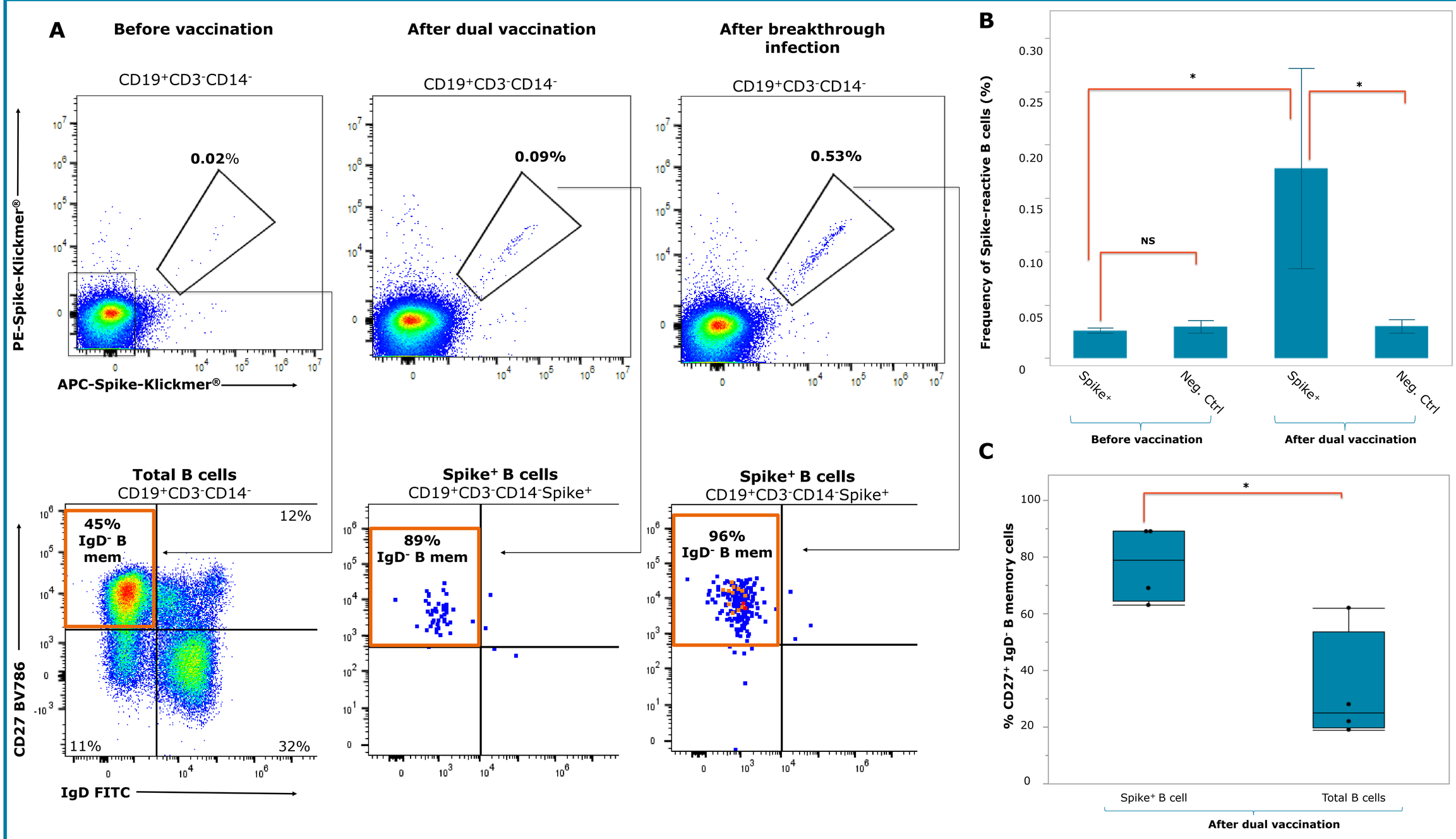

Fig. 2. Detection of SARS-CoV-2 Spike-specific B cells. (A) Detection and characterization of Spike-reactive CD19+ B cells for a longitudinally sampled donor (before vaccination, after dual vaccination, and after breakthrough infection). Spike-reactive B cells are shown by using the Spike-Klickmer® assay. Spike-reactive B cells were detected using double discrimination by staining with PE-Spike-Klickmer® and APC-Spike-Klickmer® in the same staining tube. Percentages indicate the proportions of Spike-reactive cells within total CD19+ B cells. A Spike response was detected after the dual dose of vaccine and was significantly boosted the breakthrough infection. The Spike-reactive B cells were shown to predominantly have a CD27+IgD- class-switched memory phenotype. **(B)** Box plot showing frequencies of Spike-reactive B cells for the four donors before and after vaccination. As a reference, the PBMCs were stained with a universal negative control Dextramer reagent. **(C)** Frequencies of CD27+IgD- B memory cells within total CD19+ B cell population and Spike+ B cell population. The frequency of CD27+IgD- memory cells was significantly increased in the Spike+ B cell population compared with the total B cell population. Significant differences are depicted with asterisks.

Conclusions

- A rapid Spike-Klickmer® assay was developed and applied for detection and characterization of Spike-specific B cell response following SARS-CoV-2 vaccination.
- A Spike-specific B cell response was detected in all donors upon dual vaccination against SARS-CoV-2 with a Spike-based vaccine.
- Spike-specific B cells comprised significantly larger proportions of CD27+IgD- memory cells compared with the total population of B cells.
- The study demonstrates that the Spike-Klickmer® assay is a rapid assay for monitoring the durability and immunophenotype of SARS-CoV-2-specific B cells upon vaccination or infection without any need for upstream processing of the PBMCs such as stimulation, expansion, enrichment.