Single-Cell Multi-Omics Analysis of the SARS-CoV-2 Immune Response Using U-Load dCODE Dextramer®

Kivin Jacobsen[1], Mohammad Salem[1], Amir Ameri[1], Dilek Inekci[1], Charlotte Halgreen[1] and Liselotte Brix[1]

[1]Immudex, Virum, Denmark

Introduction

Understanding T- and B-cell immunity is important for vaccine development. Here we used the BD Rhapsody™ Single-Cell Analysis system and dCODE Dextramer® technology to study virus-specific cellular immunity in a COVID-19 convalescent individual. A hPBMC sample was analyzed with a panel of MHC I dCODE Dextramer® reagents displaying peptides from SARS-CoV-2 and other viruses. Using the U-Load® technology, peptides were first loaded onto easYmers® MHC complexes, then attached to U-Load dCODE Dextramer®. The MHC I dCODE Dextramer® panel identified virus-specific T cells and their TCR clonotypes and was used together with BD AbSeq™ Immune Discovery Panel for deep phenotyping, and targeted BD Rhapsody™ Immune Response Panel for targeted mRNA expression.

We were able to identify SARS-CoV-2-specific T-cells based on TCR recognition and found the TCR clonotype and phenotype of the individual clones of spike and non-spike-specific CD8+ T cells. Our technology provides resource for studying the immunology of infectious disease and adds to the development of effective therapeutic vaccine strategies, by means of identifying and understanding specific immune cells in vaccinated and or previously infected individuals.

Versatile Workflow: SARS-CoV-2 T-Cell Detection: U-Load dCODE Dextramer® (RiO) and BD Rhapsody® Single-Cell Analysis System

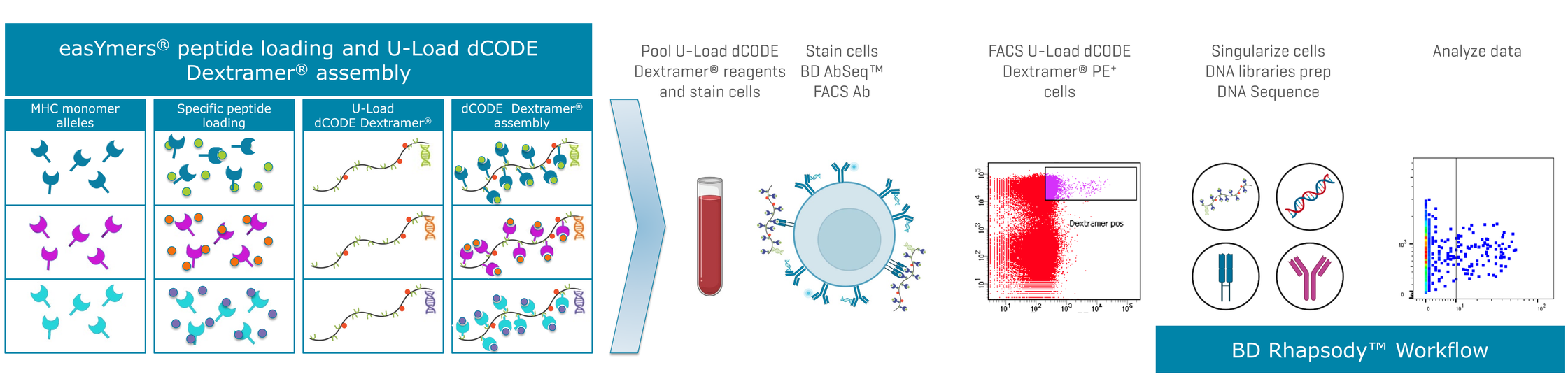
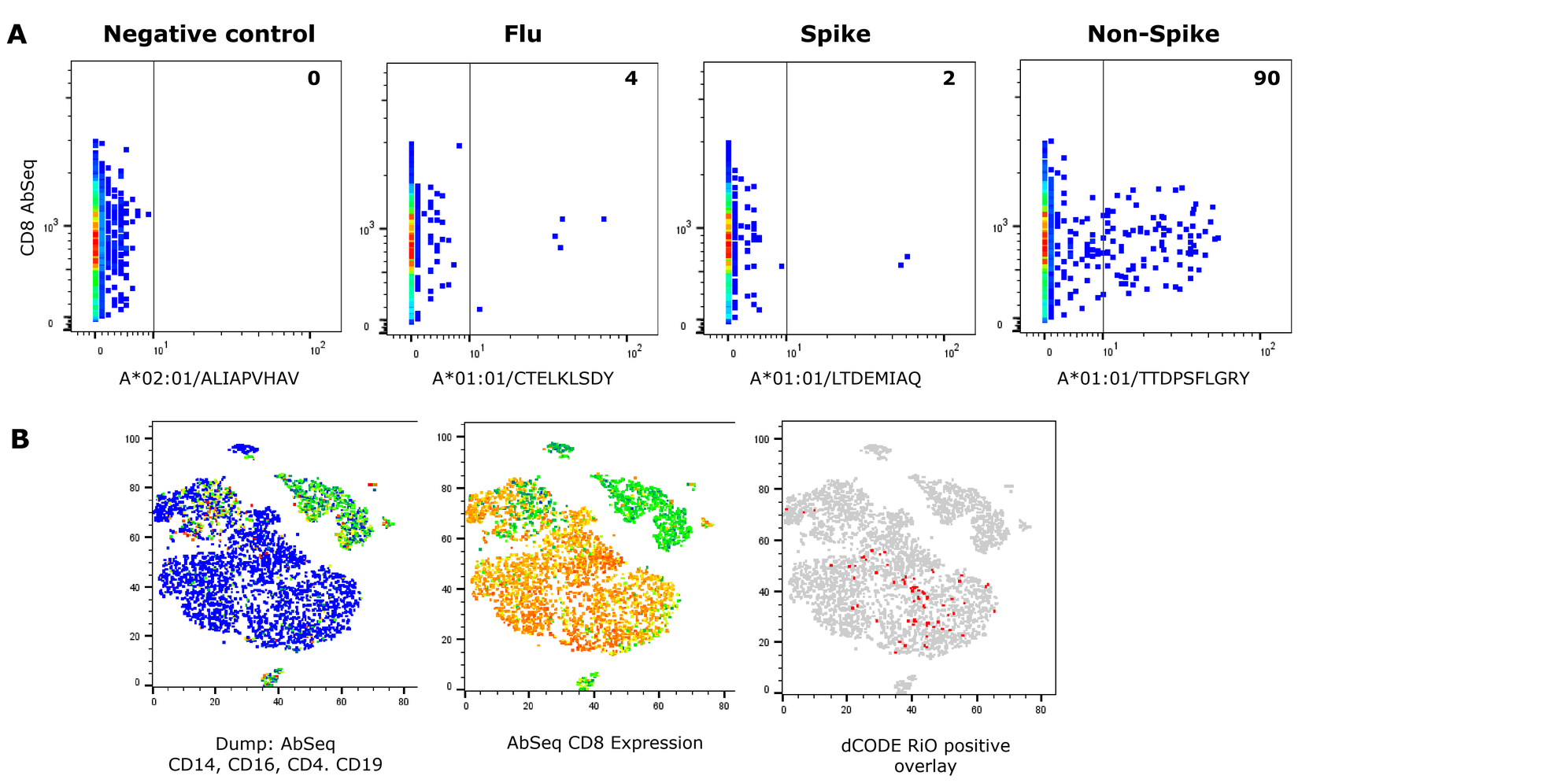


Figure 1. U-Load dCODE Dextramer® reagents were prepared by combining peptides, easYmers® and U-Load dCODE Dextramer®. 18 peptides were derived from the SARS-CoV-2, spike, nucleocapsid phosphoprotein and non-structural proteins (ORF1ab, ORF3a and ORF7a). Additional, 7 viral-specific peptides (EBV, CMV and Flu) were included along with 3 negative control peptide. Peripheral blood mononuclear cells (PBMCs) from a COVID-19 convalescent donor carrying the HLA-A*01:01, B*07:02 and B*44:02 alleles were collected and stained with this pool of 28 U-Load dCODE Dextramer® (RiO) reagents and co-stained with 30 immunophenotyping BD AbSeq™ Immune Discovery Panel for sequencing-based protein detection and fluorochrome conjugated antibodies, for cell sorting. To ensure sufficient cell numbers of antigen-specific T cells, Dextramer-positive T cells were enriched by fluorescence-activated cell sorting (FACS) before using the BD Rhapsody™ single cell analysis system for single cell capture. Specific primer-pairs were used to generate the TCR, BD AbSeq™ and dCODE® RiO libraries. For mRNA library construction, we used the BD Rhapsody™ Immune Response Targeted Panel for Human, consisting of primer sets for amplification of 399 genes. Sequencing was performed using Illumina sequencing platform. The fastq files were processed using BD Rhapsody™ Analysis Pipeline and the data analyzed using SeqGeq™. A total of 5,300 single cells were recovered after sequencing.

Characterization of SARS-CoV-2 hPBMC and SARS-CoV-2 Antigen-Specific T Cells



C

Clonotypes (beta chains)

Specificity	CDR3 Sequence	# of cells
Flu	AISEGSDPAYEQY	4
Spike	GIPGDLWPGELF	2
Non-Spike	ASSHTPGGMTYEQY	3
	ATTPTARGELF	3
	ASRPTGTGELF	2
	ASVVGYSTDTQY	2
	ASSLSGGVVNEQF	2
	ASSPTHPVYNEQF	2

D

Non-Spike T-cell Phenotyping

Surface marker	Status
CD45Ra	+
CD27	+
CD127	+
CCR7	-
CD28	+

Figure 2. Identification of antigen-specific T cells in COVID-19 PBMC sample. A) We deconvolved the antigen-specific T-cell responses to 3 distinct viruses. Among these responses, A*01:01/TTDPSFLGRY (non-spike protein SARS-CoV-2) elicited the strongest response. However, we also observed a response to A*01:01/LTDEMIAQ (spike protein) and to A*01:01/CTELKLSDY (influenza (flu)). Threshold was set based on the negative control. **B)** t-SNE plots, overlayed with AbSeq antibody expression to showing the main phenotype of the sorted cells, and overlay of dCODE® RIO positive cells which, exclusively overlay with the CD8 high expressing population **C)** To further assess the Dextramer- positive T cells; we considered the assignment of individual cells to the most abundant T cell clones. For the CTELKLSDY and LTDEMIAQ epitope, all cells matched a single clonotype. For the TTDPSFLGRY epitope clonotypes were scattered among multiple cells with the two most dominant TCR clonotypes assigned to 3 cells each. **D)** Based on the expression of numerous surface markers in the BD AbSeq™ Immune Discovery Panel, the phenotypic status of the COVID-19 and Flu antigen specific T cells, were found to be naïve memory T cells (data not shown).

Conclusion

- We were able to detect virus specific T cells with a number of different clonotypes: 4 Flu specific T cells of same beta chain clonotypes, as well as antigen- specific T cells clones against 1 SARS-CoV-2 Spike peptide and 6 different non-spike clonotypes (figure 2C), indicating a larger non-spike than Spike T- cell response in this individual.
- The main phenotype of the T-cell response were defined by surface markers, as being CD45Ra, CD27, CD28 and CD127 positive, CCR7 negative, indicating an effector memory like phenotype.
- These data demonstrate how dCODE Dextramer® can decipher virus-specific cellular immunity in a previously infected individual. The technology and method may also be used to evaluate T-cell responses after vaccination, and to access the specificity, magnitude and efficiency of a vaccine.

MM0228.01. For research use only. Not for use in diagnostic or therapeutic procedures.
Immudex®, Dextramer®, dCODE®, U-Load dCODE Dextramer® (RiO) are trademarks owned by Immudex.
The easYmers® technology is powered by immunAware. BD, BD Rhapsody and AbSeq are trademarks or registered trademarks of Becton, Dickinson and Company or its affiliates.