Discovery of antigen-specific B-cell and T-cell clonotypes using a multiplexed dCODE Dextramer®-based workflow

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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 DNA barcoded dCODE Dextramer® and dCODE Klickmer® reagents allow detection of antigen-specific cells through their corresponding B and T cell receptors (BCR/TCR), providing access to full-length paired receptor sequences, gene expression profile, and cell surface proteins from the same cell when used in combination with single cell technologies. Obtaining full-length, paired BCR sequences provides a resource for efficient antibody discovery for therapeutic and prophylactic use. Likewise, accessing full-length, paired TCR sequences enables investigation of receptor potential in adoptive cell therapies like CAR T-cell therapy.

Here, we established a workflow for simultaneous discovery of antigen-specific B-cell and T-cell clonotypes using a multiplexed dCODE Dextramer®-based approach combined with the 10x Chromium Single-Cell Analysis System.

Workflow for simultaneous investigation of antigen-specific B and T cells in PBMC samples

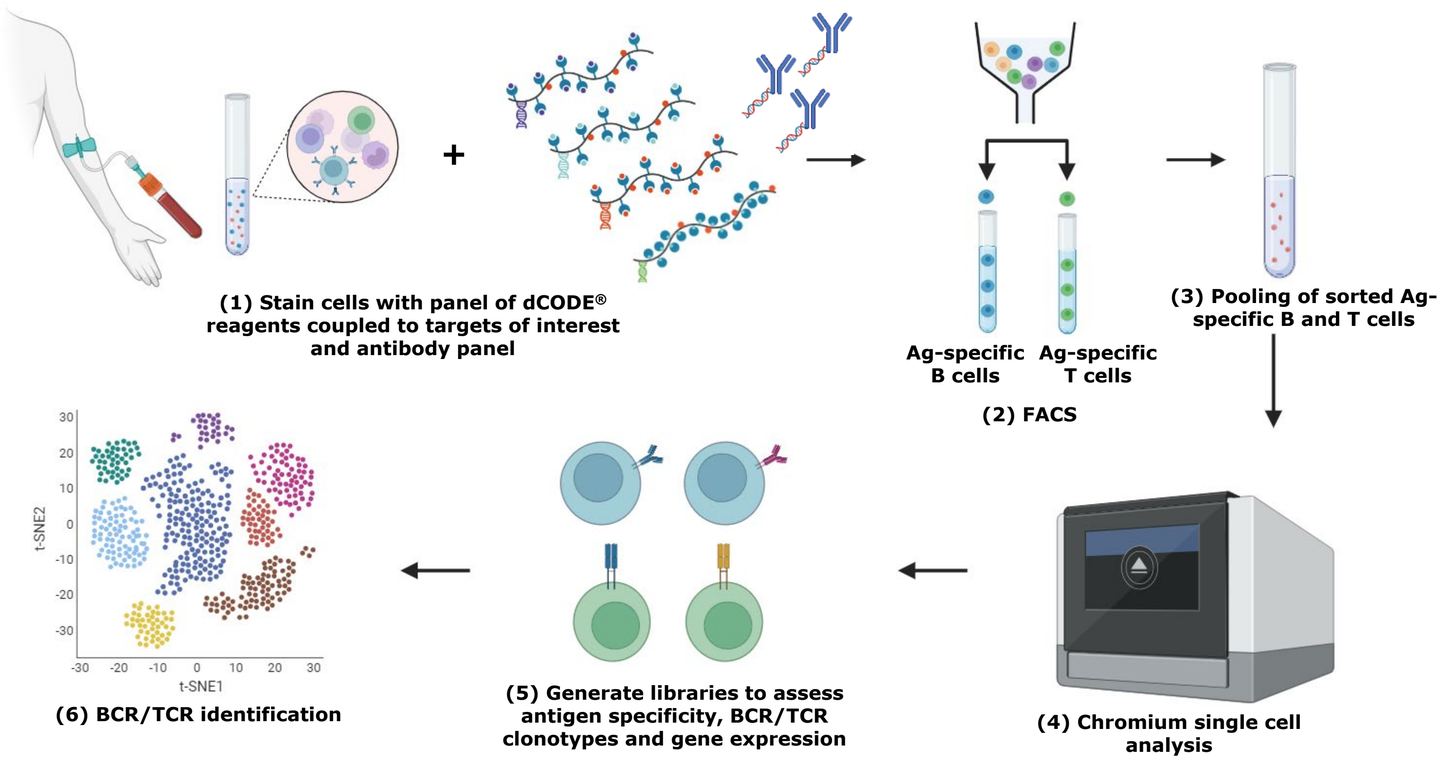
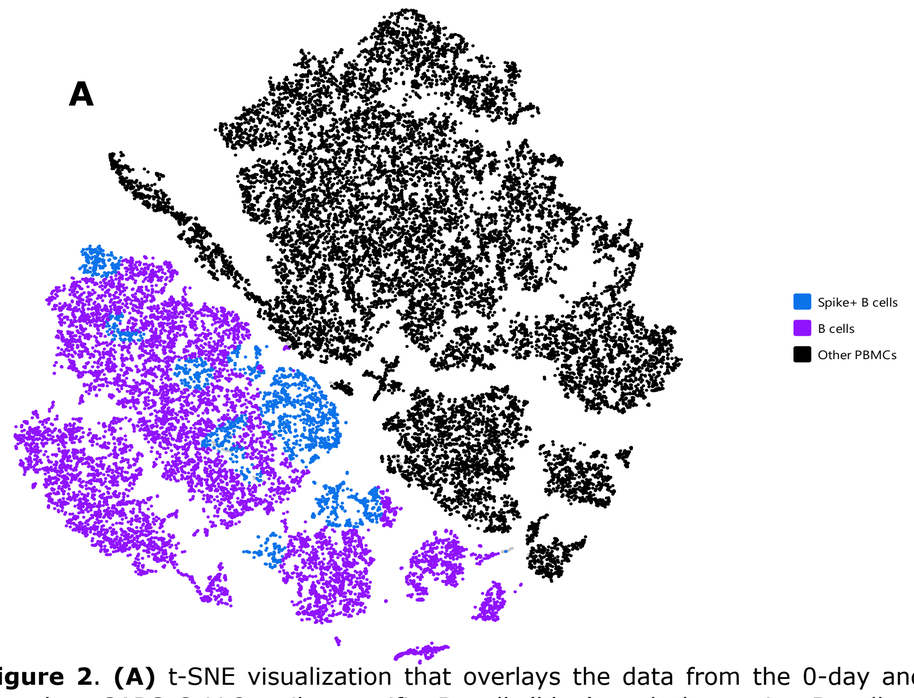


Figure 1. Experimental overview. A virus-specific model was used to establish the workflow containing a panel of dCODE MHC Dextramer® and dCODE Klickmer® reagents covering 21 specificities for detection of antigen-specific B and T cells, respectively, recognizing **CMV**, **EBV**, **flu**, **SARS-CoV-2** antigens and controls. The workflow was demonstrated by assessing the virus specific cells and their BCR/TCR clonotypes after a SARS-CoV-2 infection. PBMC samples were collected from a donor immediately after (0-day) and six weeks after (6-week) infection. Each of the PBMC samples was **(1)** stained with the panel of dCODE® reagents in the same tube and subjected to **(2)** 2-way sorting of antigen-specific cells by flow cytometry to sort Ag-specific B and T cells, respectively, **(3)** pooling of sorted Ag-specific B and T cells, **(4)** 10x Chromium Single-Cell Analysis System, **(5)** Library preparation and sequencing and **(6)** BCR/TCR identification.

Divergences in the BCR repertoire shortly following infection versus six weeks post-infection



B

Differences in surface marker profile 6-week vs. 0-day samples		
Gene symbol	**P value**	**Regulation**
CD27	1.69e-175	Up
IgD	8.13e-14	Down
Spike dCODE Klickmer®	1.80E-17	Up

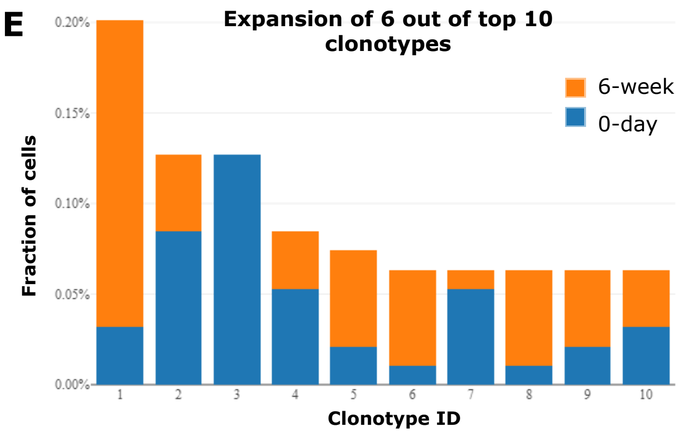
C

Subset of enriched gene captures in spike-specific B cells vs. non-spike B cells		
Gene symbol	**P value**	**Regulation**
CRIP1	4.88e-9	Up
IFI30	9.29e-8	Up
PLAC8	3.47e-3	Up
SUB1	3.68e-3	Up
S100A10	4.87e-3	Up
CIB1	4.79e-3	Up
GAPDH	3.33e-2	Up

D

BCR Clonotypes		
Origin	**Cell no.**	**Clonotypes**
0-day	4685	4469
6-week	4765	4487

E

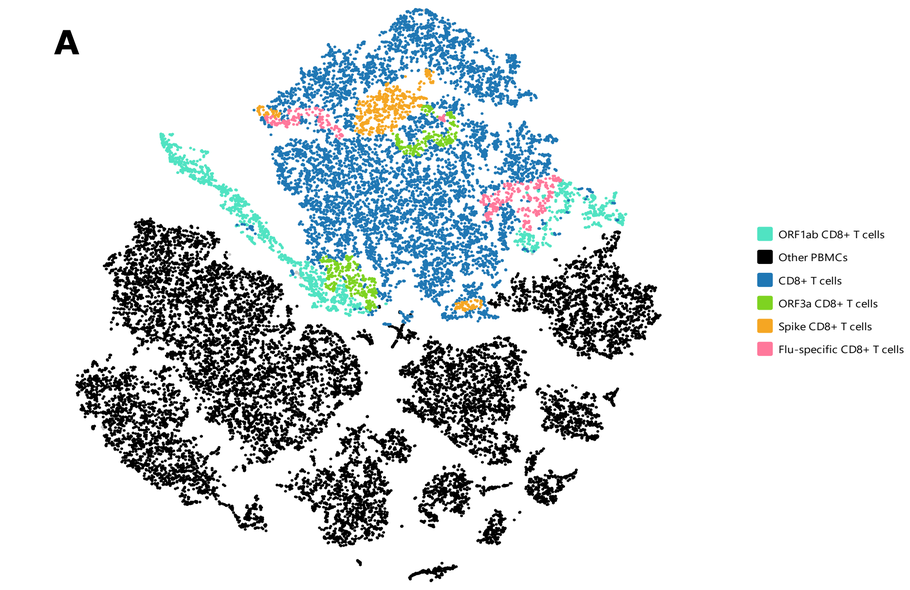


F

Top 5 Clonotype CDR3 Sequences		
Clonotype	**CDR3s**	**Frequency**
1	IGH:CTTPNMGYW IGK:CMQGTHWPYTF	19 (0.2%)
2	IGH:CARGIRGGTYYGVDYFDIW IGK:CQQYVSLPWTF	12 (0.13%)
3	IGH:CARCAGGDCYSTPSASW IGH:CARDQVVERRGIDYW IGL:CQVWDSSTVVF	12 (0.13%)
4	IGH:CARGVWSTRDTAYYLDYW IGK:CQQYNSYPQTF	8 (0.08%)
5	IGH:CVKDEYEFGHDAFDKW IGK:CQQYGGPSYAF	7 (0.07%)

Figure 2. **(A)** t-SNE visualization that overlays the data from the 0-day and 6-week samples. SARS-CoV-2 spike-specific B cell (blue) and the entire B cell population (purple) are highlighted. **(B)** The 6-week samples showed a significantly increased number of spike-specific B cells and a class-switched memory phenotype (IgDneg, CD27pos). **(C)** We identified several changes in the gene expression profile when comparing the spike-specific B cell with the remaining B cell population. **(D)** A total of 4685 (0-day) and 4765 (6-week) B cells were called in the VDJ pipeline with a high frequency of productive V-J spanning pairs (92%). We detected 4469 and 4487 BCR clonotypes, indicating a high BCR clonotype diversity. **(E, F)** 6 out of 10 top clonotypes showed an expansion at week 6.

Divergences in the TCR repertoire shortly following infection versus six weeks post-infection



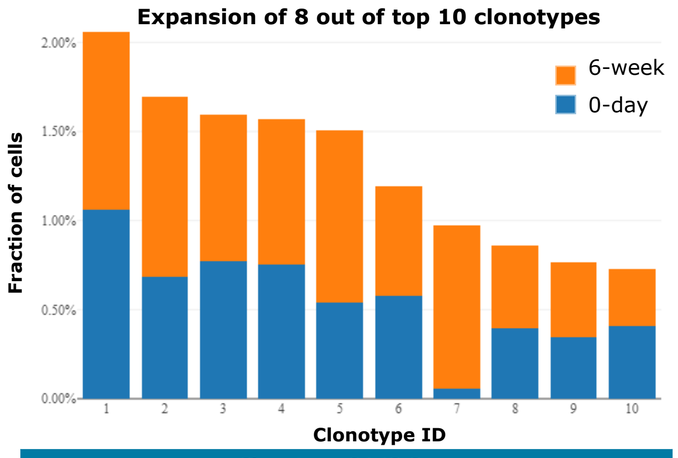
B

Subset of enriched gene captures in ORF1ab CD8 T cells vs. other CD8 T cells		
Gene symbol	**P value**	**Regulation**
STMN1	4.57e-63	Up
TUBA1B	6.42e-51	Up
HIST1H4C	4.97e-42	Up
HMGB2	6.11e-31	Up
TUBB	2.05e-28	Up
H2AFZ	2.21e-26	Up
LGALS1	1.29e-22	Up

C

TCR Clonotypes		
Origin	**Cell no.**	**Clonotypes**
0-day	3382	2179
6-week	5659	3173

D



E

Top 5 Clonotype CDR3 Sequences		
Clonotype	**CDR3s**	**Frequency**
1	TRB:CASSPQVGDEQYF TRA:CAMRDLYNFNKFYF	186 (2%)
2	TRB:CASSFEGTSDEQFF TRA:CALSKYNQGGKLIF	153 (1.7%)
3	TRB:CASSIRVGYRPYEQYF TRA:CAMSLGTGANNLFF	144 (1.6%)
4	TRB:CASSDSQHYEQYF TRA:CVVRGGGYNKLIF	142 (1.57%)
5	TRB:CASSVEGSGANVLTF TRA:CASKGGGKLIF	136 (1.5%)

Figure 3. **(A)** t-SNE visualization that overlays the data from the 0-week and 6-week samples. SARS-CoV-2 ORF1ab-specific CD8+ T cells were shown to be present in a high number (turquoise). **(B)** This population showed a significantly different gene expression profile compared with the remaining CD8 T cell population. **(C)** A total of 3408 (0-day) and 5680 (6-week) T cells and 3382 (TP1) and 5659 (TP2) B cells were called in the VDJ pipeline with a high frequency of productive V-J spanning pairs (72%). **(D)** There was an expansion of 8/10 top 10 clones at week 6 with **(E)** the top 5 clonotypes constituting 8% of the identified TCR clonotypes.

Conclusion

(1) We have demonstrated a multiplexed workflow for simultaneous detection of Ag-specific B and T cells in blood samples also providing information on their corresponding BCR and TCR clonotypes, gene expression and surface marker profiles

(2) We included a total of 21 antigen specificities using a virus-based model system

(3) The workflow can be tailored to cancer-specific responses by using dCODE® reagents displaying cancer antigens

(4) The workflow allow characterization of disease-specific immune responses with potential to facilitate diagnostic approaches, prediction of disease progression and development of novel cancer vaccines and therapeutics

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