MHC II dCODE Dextramer technology allows characterization of antigen-specificity, TCR clonotype and gene expression of single CD4+ T-cells

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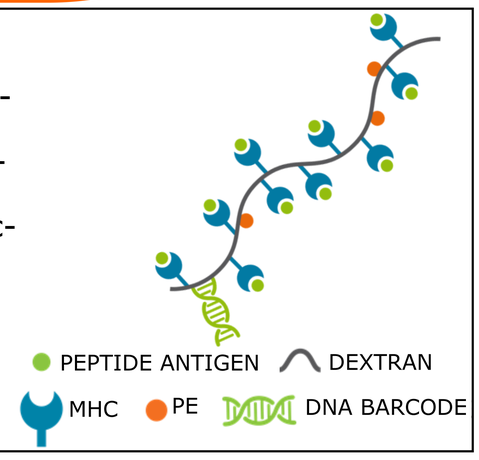
Background

T-cell mediated adaptive immunity to pathogens, tumor- and self-antigens in autoimmunity is dependent on the specific recognition of the peptide-MHC (pMHC) complex on the target cell by a unique T-Cell receptor (TCR). Identifying the specific T-cells, their phenotypic gene expression profiles and clonotypic TCR sequence is essential to understand the complexity of an immune response and to manipulate it for therapeutic benefits. We have developed an MHC multimer technology, dCODE™ Dextramer®, comprising a dextran backbone displaying multiple pMHCs and a unique DNA barcode coding for the displayed pMHC specificity. Detection of cells labelled with dCODE Dextramer is based on sequencing of the attached DNA barcode. The dCODE Dextramer is compatible with 10x Genomics Chromium platform for single cell analysis.
In this study, a panel of three different MHC II dCODE Dextramer reagents was used to identify antigen-specific CD4+ T-cells in a cell sample, together with their unique TCR clonotypes and gene expression profiles.
Two workflows were compared. In one, cells stained with dCODE Dextramer were directly used to perform single cell analysis, while in the other, stained cells were previously enriched for antigen specific T-cells by cell sorting.



Workflow and Results

Cell labelling reagents

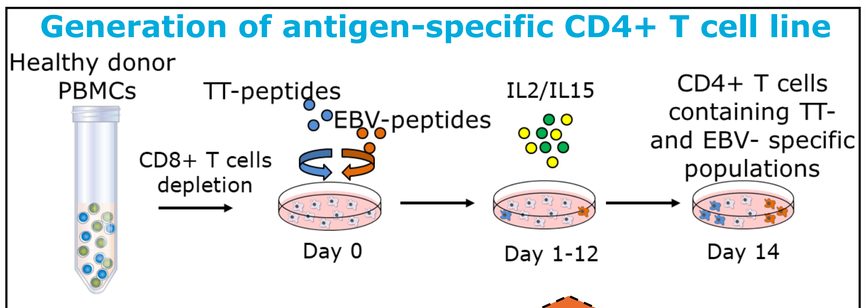
- MHC II dCODE Dextramer reagents:
  1) DR1/EBV: DRB1*0101/TSLYNLRRGTALA (Epstein Bar Virus specific)
  2) DR1/TT: DRB1*0101/KIYSYFPSVISKV (Tetanus Toxoid specific)
  3) DR1/CLIP: DRB1*0101/ PVSKMRMATPLLMQA (negative control)
- Feature Barcoded antibody (BioLegend): TotalSeq-anti-CD3
- Fluorochrome labelled antibodies for cell markers (BioLegend):
  CD3/FITC
  CD4/PerCPCy5.5
  CD8/Pacific Blue

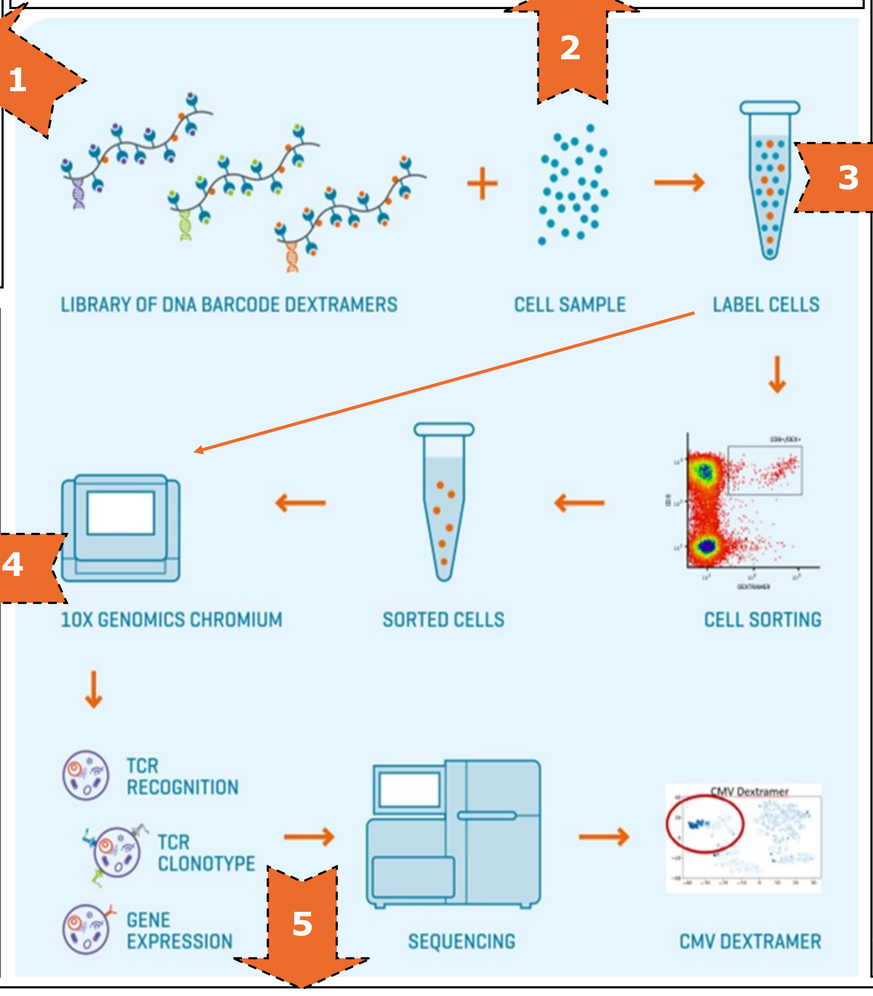
dCODE Dextramer technology combined with Feature Barcoding

- CD4+ T-cell sample (box 2) was stained with the panel of dCODE Dextramer reagents, TotalSeq-anti-CD3 and fluorchrome labelled antibodies (box 1).
- Stained cells were either subjected to Chromium System for single cell analysis using Feature Barcoding technology, or sorted by flow cytometry before undergoing single cell analysis.
- In both procedures, three DNA libraries were generated:

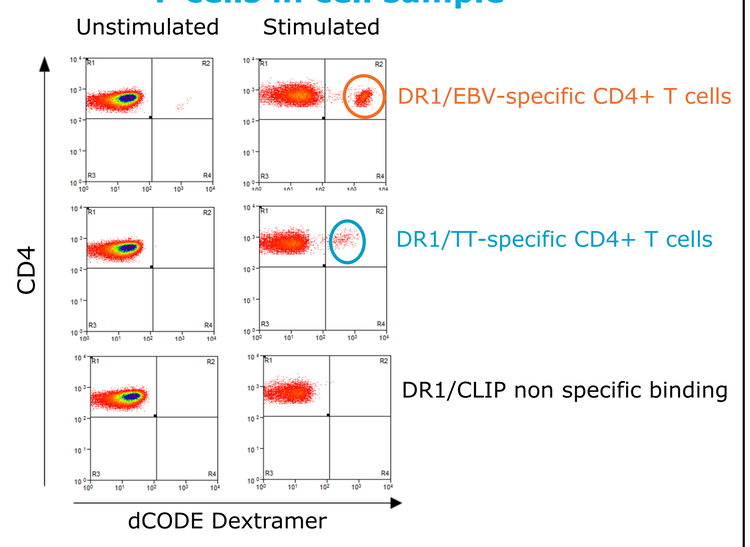
  1)Surface markers library (dCODE Dextramer reagents and TotalSeq CD3 antibody)
  2)TCR repertoire library
  3)Gene expression library

Generation of antigen-specific CD4+ T cell line

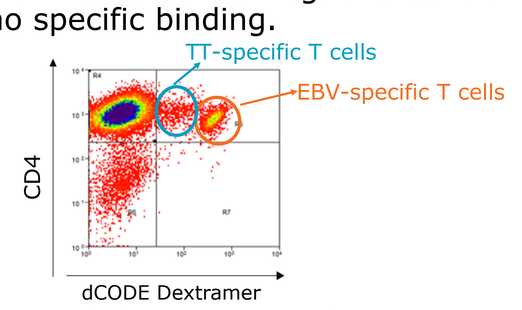




MHC II dCODE Dextramer detect specific T-cells in cell sample



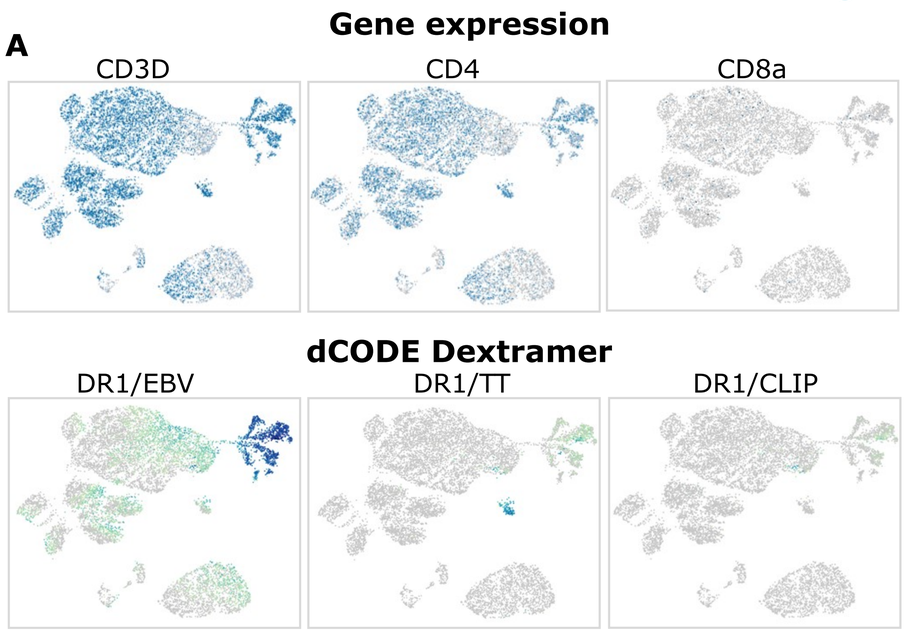
A) DR1/EBV or DR1/TT dCODE Dextramer were used to detect antigen-specific CD4+ T-cells in a cell line stimulated with EBV and TT peptides. Few or no antigen-specific CD4+ T-cells were detected in the unstimulated cell line. DR1/CLIP dCODE Dextramer was used as negative control and detected no specific binding.



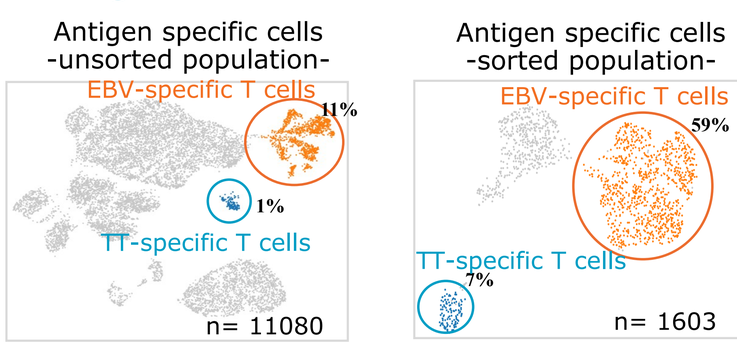
B) Staining of the stimulated cell sample with a pool of DR1/EBV, DR1/TT, and DR1/CLIP dCODE Dextramer detected two distinguishable antigen-specific populations, corresponding to EBV- and TT-specific CD4+ T-cells in the cell sample.

Characterizing antigen-specific CD4+ T-cells using MHC II dCODE Dextramer

A



B



C

EBV-specific TCR clonotypes-unsorted population / EBV-specific TCR clonotypes-sorted population

clonotypes	TRA CDR3	TRB CDR3	# cells	TRA CDR3	TRB CDR3	# cells
1	TGTGCTCTCTATAACACCAATGCAGGCAAATCAACCTTT	TGCAGCGTTGATCGGGGGGTTGGCTATGAGCAGTTCTTC	491	TGTGCTCTCTATAACACCAATGCAGGCAAATCAACCTTT	TGCAGCGTTGATCGGGGGGTTGGCTATGAGCAGTTCTTC	527
2	TGTGCCGTCTATAACACCAATGCAGGCAAATCAACCTTT	TGCAGCGTTGAATCGGCAGGGGCAGGGGATACGCAGTATTTT	50	TGTGCCGTCTATAACACCAATGCAGGCAAATCAACCTTT	TGCAGCGTTGAATCGGCAGGGGCAGGGGATACGCAGTATTTT	13
3	TGTGCTCTCTATAACACCAATGCAGGCAAATCAACCTTT	TGCAGCGCTGAAGAGGCGGGGGAGCGGGGACGAGCAGTACTTC	17	TGTGCTCTCTATAACACCAATGCAGGCAAATCAACCTTT	TGCAGCGCTGAAGAGGCGGGGAGCGGGGACGAGCAGTACTTC	11

TT-specific TCR clonotypes-unsorted population / TT-specific TCR clonotypes-sorted population

clonotypes	TRA CDR3	TRB CDR3	# cells	TRA CDR3	TRB CDR3	# cells
1	TGTGCAATGAGAGGCATACTCACCGGTAACCAGTTCTATTTT	TGCAGTGCTAAATCCCCGGGACAGGGTTATGAAAAACTGTTTTTT	28	TGTGCAATGAGAGGCATACTCACCGGTAACCAGTTCTATTTT	TGCAGTGCTAAATCCCCGGGACAGGGTTATGAAAAACTGTTTTTT	33
2	TGTGCAGTGTCCGGGGGGTCTGGTTCTGCAAGGCAACTGACCTTT	TGTGCCAGCAGCTTAGACGGCCGAGGGGGGGGCTACACCTTC	17	TGTGCAGTGTCCGGGGGGTCTGGTTCTGCAAGGCAACTGACCTTT	TGTGCCAGCAGCTTAGACGGCCGAGGGGGGGGCTACACCTTC	17
3	TGTGCTGTGGGCAACGACTACAAGCTCAGCTTT	TGTGCCAGCAGCAGAACGCCAGATACGCAGTATTTT	9	TGTGCAGTGTCCGGGGGGTCTGGTTCTGCAAGGCAACTGACCTTT	TGTGCCAGCAGCTTAGACGGCCGAGGGGGGGGCTACACCTTC	14

A) The three DNA libraries were sequenced by Illumina NGS sequencing. Sequencing data were analyzed using Cell Ranger analysis, and results visualized by Loupe Cell Browser. CD4+ T-cells were clustered and classified based on Gene expression and dCODE Dextramer. Clusters of EBV– and TT– specific T-cells were detected by specific dCODE Dextramer.

B) EBV– and TT– specific T-cells were identified in both unsorted and sorted CD4+ T-cell populations.

C) The top 3 identified EBV– and TT–specific TCR clonotypes were conserved across unsorted and sorted CD4+ T-cell populations. For each clonotype, the corresponding number of cells is reported.

Conclusion

In this study we have:

- Set up a protocol to stimulate antigen-specific T cell response in a CD4+ T cell population.
- Identified antigen-specific T-cell populations in the CD4+ T cell sample.
- Identified TCR clonotypes in the antigen-specific populations, and sequenced the relative VDJ combinations, using MHC II dCODE Dextramer in combination with the Feature Barcoding technology.

For the first time, DR1-restricted TCR clonotypes have been identified in EBV– and TT-specific T cell populations.
The identification of novel MHC II-restricted TCR sequences offers the possibility to progress immunological research and immunotherapeutic development.

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