Dissecting MAIT cell phenotype and TCR sequences using MR1 reagents and single-cell RNA sequencing

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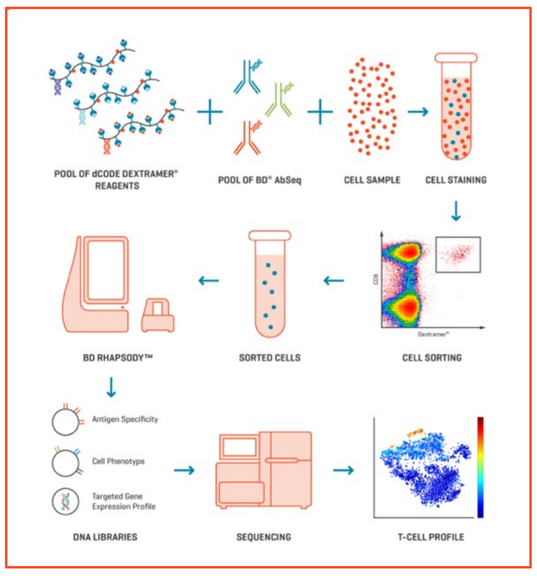
Introduction

Mucosal-associated invariant T (MAIT) cells constitute a large and specialized subset of T cells with both innate and adaptive features. Unlike conventional T cells, MAIT cell TCRs exhibit reduced sequence variability and target metabolites of both bacterial and endogenous origin in the context of the MHC-I-like molecule known as MR1. To help study MAIT cells we have developed MAIT cells specific reagents, MR1 dCODE Dextramer®, and demonstrate here:

- a workflow, which enables the study of MAIT cell phenotypic markers combined with TCR sequencing using MR1 dCODE Dextramer® reagents on the BD Rhapsody™ Single-Cell Analysis System.
- that recombinantly expressed TCRs from identified MAIT TCR sequences specifically bind to their antigen, MR1/5-OP-RU, in a bead-based assay.

Workflow of the full immunological profiling of MAIT cells



The full immunological profiling of immune cells in a single workflow by combining dCODE Dextramer® reagents and the BD Rhapsody™ Single-Cell Analysis System.

Conclusion

- MR1 dCODE Dextramer® reagents achieve sensitive and specific detection of MAIT cells and, when used with the BD Rhapsody™ Single-Cell Analysis System, facilitate single-cell multi-dimensional analyses that reveal correlations between phenotype, TCR sequence, and gene expression.
- The MR1/5-OP-RU dCODE Dextramer® reagent reliably and specifically identified CD3+CD8+CD161high that phenotypically were memory-like (CCR7–CD45RAlowCD62Llow).
- Differential gene expression of MR1/5-OP-RU-specific MAIT cells compared to other CD3+ T cells reflected their unique response to immunological environments and signals.
- The V(D)J and CDR3 analysis confirmed the reported semi-invariant TCR α chain of MAIT cells. TRAV1-2, TRAJ33, TRBV6, and TRBV20 were the most used gene segments. Despite both α and β chains of the TCR showed restricted gene segment usage, the TCR repertoire of the MAIT cells was diverse, with no major clonotypic dominance.
- Recombinant expression of two MAIT TCRs confirmed their specificity to MR1/5-OP-RU, but also indicated an antigen independent binding to MR1.

Accurate detection of MAIT cells within the sequencing data

Figure 1

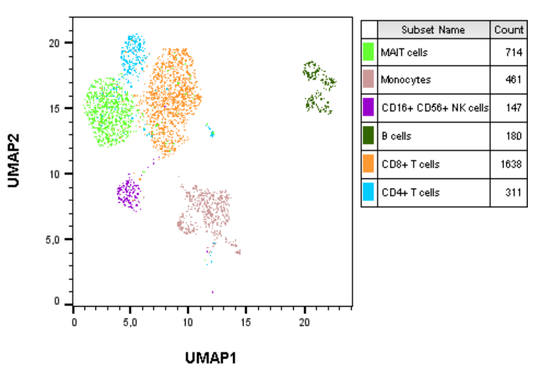


Figure 1. UMAP clustering based on gene expression data. Overlaying clusters with surface markers (BD Rhapsody™ Immune response panel antibodies and MR1 dCODE Dextramer®) identified the major cell lineages (CD4+ and CD8+ T cells, B cells, Monocytes, NK cells) and CD161^{High}+ MAIT cells among the CD3+ T cells.

Figure 2a

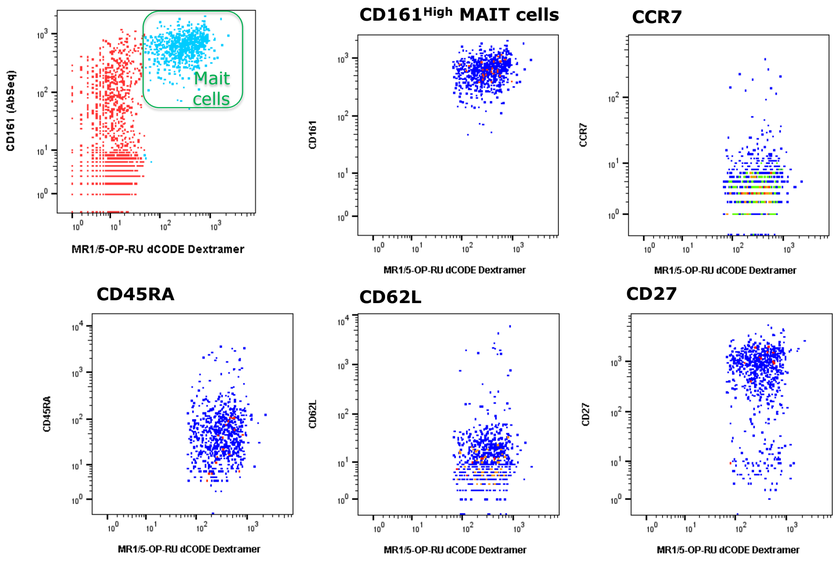


Figure 2b

Figure 2. MAIT cells exhibit a memory-like phenotype (CCR7–CD45RA$_{low}$CD62L$_{low}$) and a non-active (CD137–CD25–HLA-DR–)(not shown) and response-ready state (CD27^{high}). **a**) MAIT cells were identified by cell surface markers (CD3+CD161^{high}MR1/Dex+). **b**) Cell surface marker analysis of MAIT cells.

Figure 3

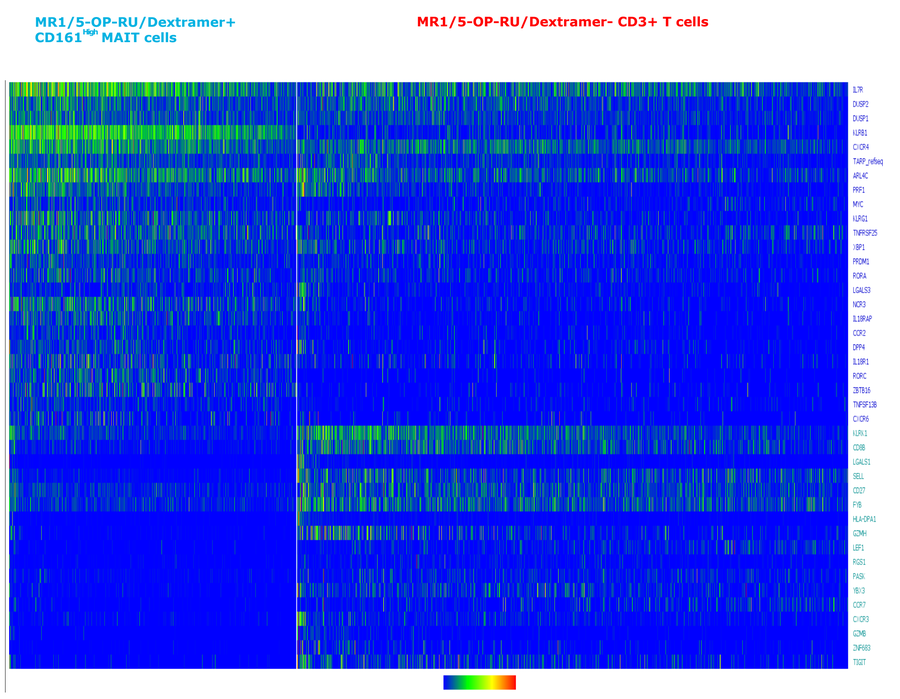
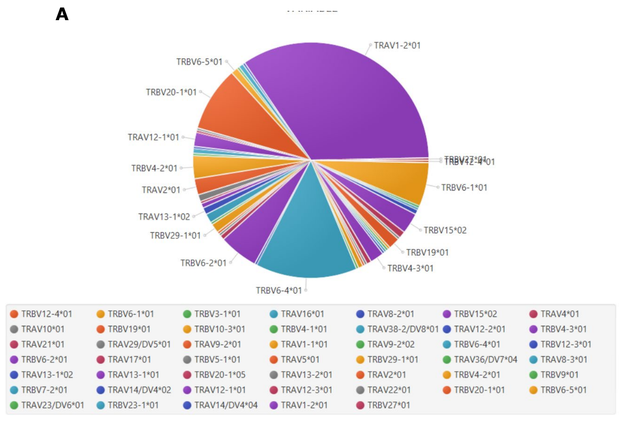


Figure 3. Differential gene expression: shows the top up- and downregulated genes identified in the MAIT cell population compared to the remaining CD3+ T cells. Several of the differentially expressed genes are immune-related, indicative of the unique response of MAIT cells to different immunological environments and signals.

V(D)J analysis of MAIT TCR shows high variation



C

TRAV	TRBV	CDR3a	CDR3b	Cells	Cell Frequency (%)
TRAV1-2*01	TRBV6-4*01	AVMDSNYQLI	ASSSGSTDTQY	5	3,2
TRAV1-2*01	TRBV6-4*01	AVRDSNYGLI	ASSSGSYNEQF	4	2,5
TRAV2*01	TRBV4-3*01	AVEDHVTNAGKST	ASSQEPSGTYNEQF	3	1,9
TRAV1-2*01	TRBV4-2*01	AVRDSNYGLI	ASSHGKLDTDTQY	3	1,9
TRAV1-2*01	TRBV20-1*01	AAMDSNYQLI	SARDVAGVTGEQY	3	1,9
TRAV1-2*01	TRBV6-1*01	AVMDSNYQLI	ASSEDAYNEQF	2	1,3
TRAV12-1	TRBV15*02	VVN*QHRQTN	ATSRDSSGANVLT	2	1,3
TRAV1-2*01	TRBV20-1*01	AVLDSNYQLI	SASRDRVSETQY	2	1,3
TRAV13-1*02	TRBV6-4*02	AASHGRRKQTH	ASSDSGTDTQY	2	1,3
TRAV1-2*01	TRBV20-1*01	AVMDSNYQLI	SAVGGLAGGPRETQY	2	1,3

Figure 4. TRAV and TRBV gene segment representation in all TCR clonotypes (**A**) and the top 10 most frequent clonotypes (**B**) of the analyzed MAIT cell population. The majority of the identified CD161+ MAIT cells (~70%) express typical TCRs consisting of TRAV1-2 and TRAJ33(not shown) as well as TRBV6 and TRBV20 gene segments. Despite the restricted gene segment usage, overall paired TCR sequence variation was high, with 138 clonotypes in 158 cells. (**C**) gene segment usage and CDR3 of 10 most frequent clones.

Validation of MAIT TCR specificity in bead-based assay

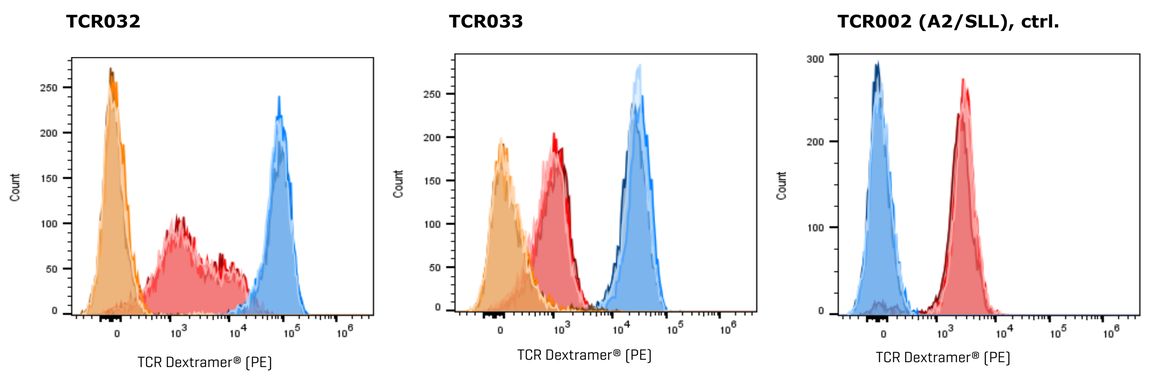


Figure 5. Recombinant TCR proteins bind specifically to MR1/5-OP-RU.

Two identified MAIT TCRs (TCR032 and TCR033) were recombinantly expressed in E.coli, refolded and evaluated for binding to their target, MR1/5-OP-RU. Both MAIT derived TCRs, but not the control TCR (TCR002), bind efficiently to MR1/5-OP-RU conjugated beads, but not to control HLA-A*0201 conjugated beads. Some binding to the MR1 loaded with control metabolite 6-FP was also observed, and is probably due to general, but weak interaction with the MR1 protein independently of the bound ligand.