Identification of functional MAIT-specific TCRs using MR1 dCODE Dextramer® reagents and evaluating antigen presentation.

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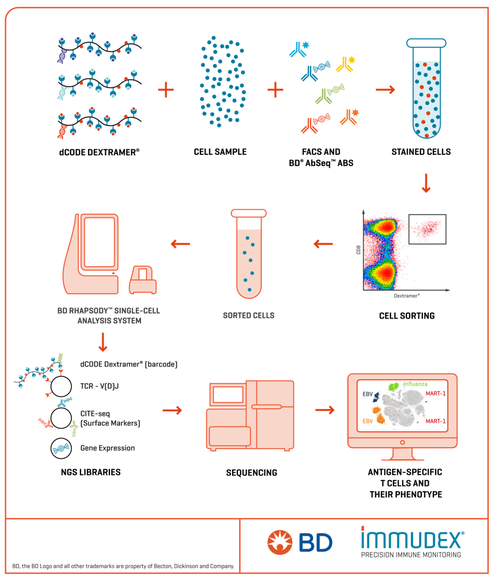
Introduction

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 achieved 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 majority of the identified CD161+ MAIT cells (~70%) expressed typical TCRs consisting of TRAV1-2 and TRAJ33 as well as TRBV6/20, TRBD1/2, and TRBJ2 gene segments (not shown) .
- Recombinant expression of two MAIT TCRs confirmed their specificity to MR1/5-OP-RU.
- We demonstrate a workflow allowing (i) Identification of MAIT cells and their corresponding TCR sequences (ii) Generation of soluble TCR molecules based on the identified sequences and validation of their specificity (iii)
- Future perspective: TCR Dextramer® displaying the MR1-specific TCRs may be used for identification of target expression on surface of cells.

Accurate detection of MAIT cells within the sequencing data

Figure 1

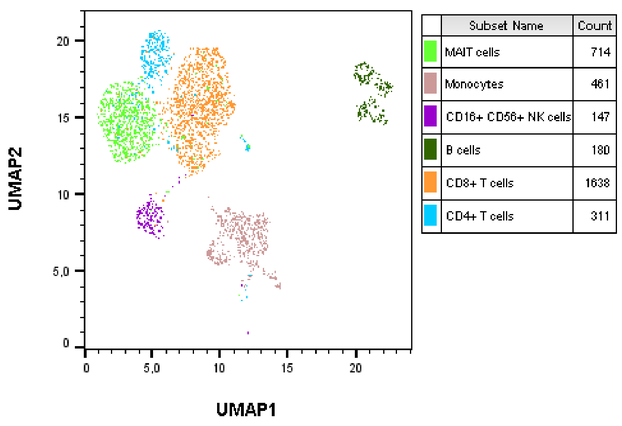


Figure 1. UMAP clustering based on gene expression data. Overlaying clusters with surface markers (BD Rhapsody™ Immune response panel antibodies and MR1 dCODE Dextramer®) identified the major cell lineages (CD4+ and CD8 + T cells, B cells, Monocytes, NK cells) and CD161^{High}+ MAIT cells among the CD3+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

Figure 2a

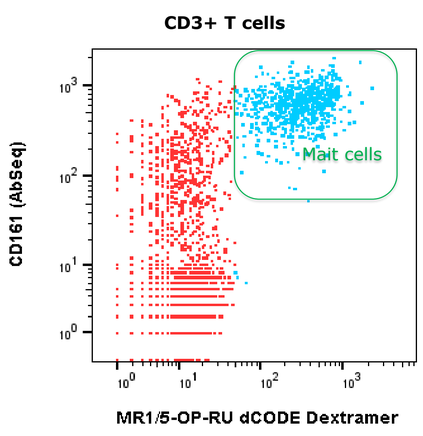
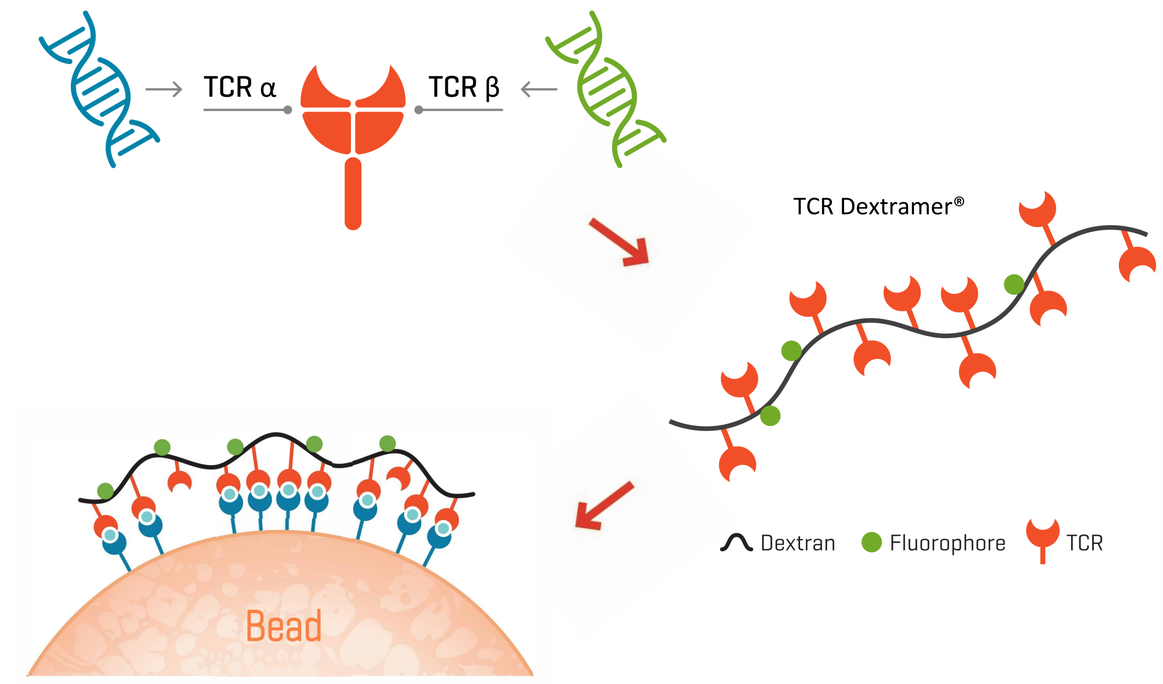


Figure 2. MAIT cells were identified by cell surface markers (CD3+CD161^{high}MR1/Dex+).

TCR Dextramer® reagents



TCR monomer alpha and beat chains were produced in *E. coli,* refolded and attached to a fluorescent Dextramer® backbone. Artificial cells (Beads coated w. target molecule) were stained with TCR Dextramer® reagents like conventional pMHC Dextramer® reagents on T cells.

V(D)J analysis of MAIT TCR and TCR monomer production

Three alpha-beta TCR sequences were selected: Two TCRs having common TRAV+TRAJ, TRBV+TRBJ combinations and one having a rare TRAV+TRAJ one (**Figure 3**). TCR alpha and beta chains were successfully expressed for all selected TCRs but only 2 TCRs showed a correctly paired TCR (**Figure 4**).

TCR ID	TRAV	TRAJ	CDR3_A	TRBV	TRBJ	TRBC	CDR3_B	Cell count (Naïve + Memory)
A	1-2*01	33*01	AVMDSNYQLI	6-4*01	2-3*01	2	ASSSGSTDTQY	3+3
B	1-2*01	12*01	AVMDSSYKLI	6-1*01	2-5*01	2	ASSELAGGQETQY	2+2
C	2*01	27*01	AVEDHVTNAGKST	4-3*01	2-1*01	1+2	ASSQEPSGTYNEQF	2+1

Figure 3. TCR-A and TCR-B have common combinations of both TRAV+TRAJ and TRBV+TRBJ. TCR-C has a rare TRAV+TRAJ but appears to have arisen independently twice due to the use of both TRBC1 and TRBC2.

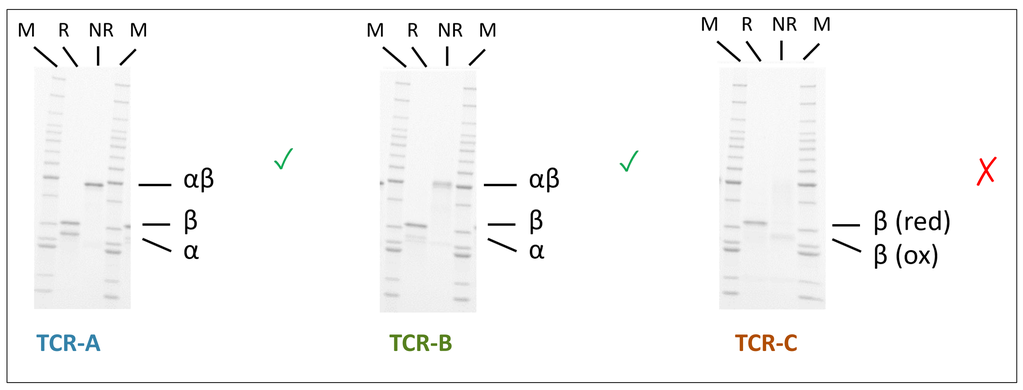


Figure 4. TCRA and TCRB were successfully produced. Visible bands corresponding to the alpha and beta chains in the reduced (R) condition. Visible band corresponding to the alpha-beta dimers in the non-reduced (NR) condition. TCR-C is incompletely refolded. Neither the alpha chain or pair alpha-beta dimers are visible in respectively reduced and non-reduced condition.

Validation of MAIT TCR specificity in bead-based assay

Based on these two TCRs (TCR-A and TCR-B), TCR Dextramer® reagents were generated and used to evaluate functionality and specificity on artificial cell (beads) surface. The two TCRs showed being correctly refolded and specific TCRs as demonstrated by proper recognition of MR1/5-OP-RU on artificial cell system.

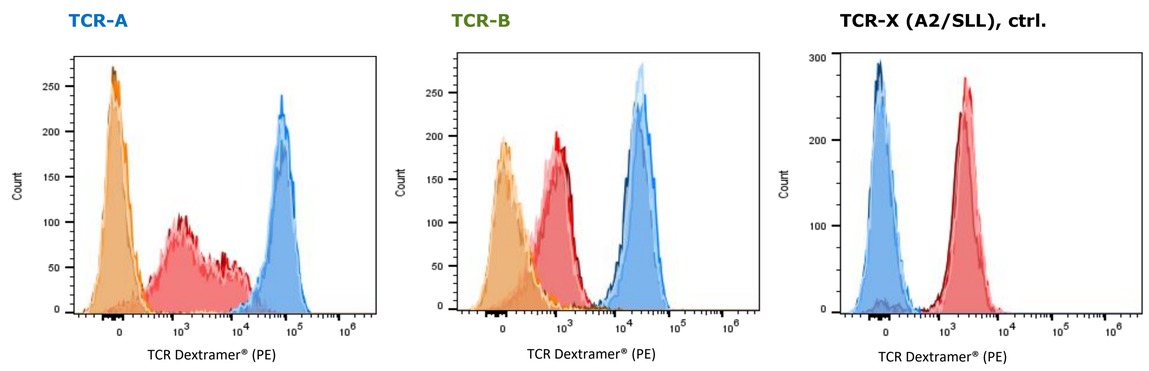


Figure 5. Recombinant TCR proteins binds specifically to MR1/5-OP-RU.

Two identified MAIT TCRs (**TCR-A** and **TCR-B**) 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 (**TCR-X**), bind efficiently to MR1/5-OP-RU conjugated beads (blue pic), but not to control HLA-A*0201 (orange pic) and not to MR1/6-FP conjugated beads (red pic).