Investigating MAIT cell phenotype using MR1 dCODE and TCR Dextramer® reagents

Thomas H. Blicher, Bjarke E. Hansen, Kivin Jacobsen, Liselotte Brix
Immudex Aps, Virum, Denmark

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 cell-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

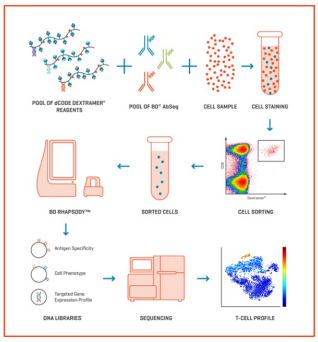

Figure 1. The full immunological profiling of immune cells in a single workflow combining dCODE Dextramer reagents and the BD Rhapsody™ Single-Cell Analysis System.

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 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, but also indicated an antigen-independent binding to MR1.
- 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) Generation of TCR Dextramer® reagents allowing identification of target expression on surface of cells.

Accurate detection of MAIT cells

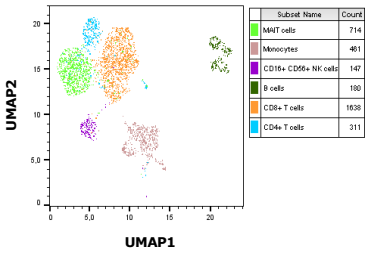

Figure 2. UMAP clustering based on gene expression data. Overlaying clusters with surface markers (BD Rhapsody™ Immune response panel antibodies and MR1 dCODE Dextramer) identified the major cell lineages (CD4+ and CD8+ T cells, B cells, Monocytes, NK cells) and CD161^{High}+ MAIT cells among the CD3+ T cells.

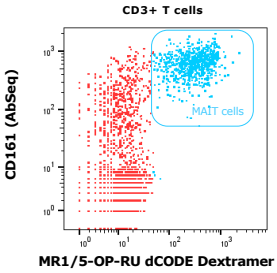

Figure 3. MAIT cells were identified by cell surface markers (CD3+CD161^{high} MR1/Dex+).

V[D]J analysis of MAIT TCR and TCR monomer production

Three αβ TCR sequences were selected: Two with common combinations of TRAV-TRAJ, TRBV-TRBJ and one having a rare TRAV+TRAJ (**Figure 4**). All TCR α and β chains were successfully expressed, but only two TCRs showed a correctly paired TCR (**Figure 5**).

TCR ID	TRAV	TRAJ	CDR3_A	TRBV	TRBJ	TRBC	CDR3_B	Cell count (Naive + 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

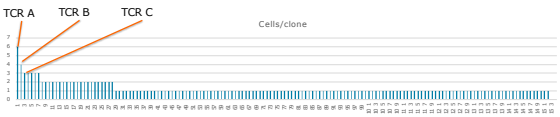

Figure 4. **TCR A** and **TCR B** common α and β chain composition. **TCR C** has a rare TRAV-TRAJ but appears to have arisen twice due to the use of both TRBC1 and TRBC2.

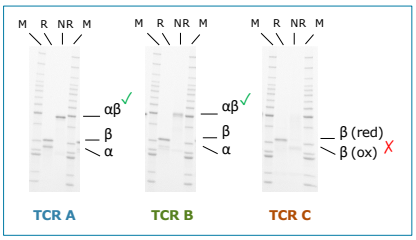

Figure 5. **TCR A** and **TCR B** were successfully produced as demonstrated by bands corresponding to the α+β chains and αβ dimers under reducing (R) and non-reducing (NR) conditions, respectively. **TCR C** appears incompletely refolded as shown by the absence of the α chain.

Validation of MAIT TCR specificity in bead-based assay

TCR Dextramer® reagents were generated based on **TCR A** and **TCR B** and used to evaluate functionality and specificity on artificial cells (beads). The two TCRs appear correctly refolded as demonstrated by specific recognition of MR1/5-OP-RU.

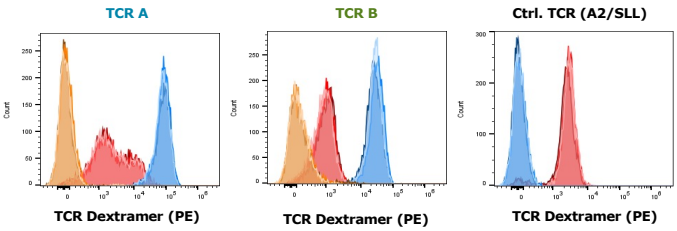

Figure 6. Recombinant TCR proteins binds specifically to MR1/5-OP-RU.
Two identified MAIT TCRs (TCR A and TCR B) were recombinantly expressed in E.coli, refolded and evaluated for binding to their target, MR1/5-OP-RU. Both MAIT-derived TCRs, but not the control TCR, bind efficiently to MR1/5-OP-RU-conjugated beads, but not to control HLA-A*0201-conjugated beads. Weak binding to MR1 loaded with the non-stimulatory metabolite 6-FP was also observed and is probably driven by weak, ligand-independent interactions between MR1 and TCR.

TCR Dextramer reagents

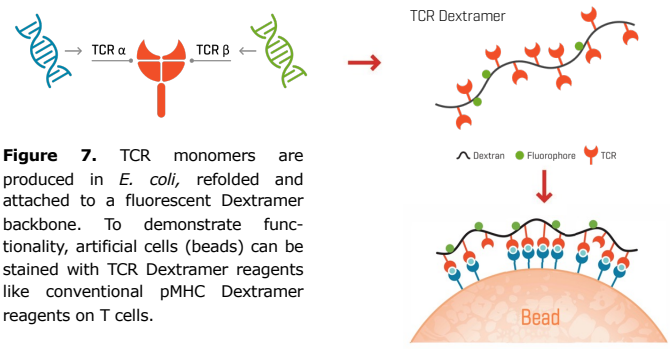

Figure 7. TCR monomers are produced in *E. coli,* refolded and attached to a fluorescent Dextramer backbone. To demonstrate functionality, artificial cells (beads) can be stained with TCR Dextramer reagents like conventional pMHC Dextramer reagents on T cells.

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