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# T cell receptor diversity, specificity and promiscuity of functionally heterogeneous human MR1-restricted T cells

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## Abstract

The monomorphic MHC-class I-like molecule, MR1, presents small metabolites to T cells. MR1 is the restriction element for microbe-reactive mucosal-associated invariant T (MAIT) cells. MAIT cells have limited TCR usage, including a semi-invariant TCR alpha chain and express high levels of CD161 and CD26. In addition to microbial lumazine metabolites, recent studies have demonstrated that MR1 is able to capture a variety of diverse chemical entities including folate-derivatives, a number of drug-like and other synthetic small molecules, and as yet undefined compounds of self-origin. This capacity of MR1 to bind distinct ligands likely accounts for the recent identification of additional, non-canonical, subsets of MR1-restricted T (MR1T) cells. These subsets can be defined based on their ability to recognize diverse microbes as well as their reactivity to non-microbial cell-endogenous ligands, including tumor-associated antigens. Herein, we will discuss our current understanding of MR1T cell diversity in terms of TCR usage, ligand recognition and functional attributes (Table I).

### Keywords

MR1; MAIT; MR1-rescticted T cells; TCR; Heterogeneity

## 1. The Paradox of the Innate-like MAIT Cells

Currently, MAIT cells are the most extensively described subset of MR1T cells and have often been described as innate-like cells. This concept was supported by their restriction by MR1, a molecule of limited polymorphism (Treiner et al., 2003), by the associated

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expression of a semi-invariant T cell receptor (TCR) comprising a nearly germline-encoded *TRAV1–2*$^{+}$ TCRα (TRAV) chain with three alternative *TRAJ* genes (namely TRAJ33, TRAJ20 or TRAJ12) and paired to a restricted set of TCRβ (TRBV) chains, (mostly TRBV6 and TRBV20; (Porcelli et al., 1993; Tilloy et al., 1999), and by their unique PLZF-driven developmental pathway (Koay et al., 2016).

Innate immunity is defined by the recognition of microbial patterns by specialized germline-encoded receptors and by the ability to mount rapid responses following stimulation. In contrast, similar to adaptive T cells, the development of MAIT cells entails the coordinated and effective pairing of somatically rearranged TRAV and TRBV chains that can interact with MR1 and presumably a ligand (Godfrey et al., 2019; Legoux et al., 2019; Legoux et al., 2020). However, and like other innate-like T cells, MAIT cells acquire effector capacities during thymic maturation, expand early in life in the periphery and express simplified TCR patterns. Accordingly, they are able to promptly respond to antigen challenge (Constantinides et al., 2019). Hence, MAIT cells share unique features with both innate and adaptive immune cells, which positions them at the crossroad of the two systems.

### 1.2 Defining features of MAIT cells

Two observations challenge the definition of innate-like T cells. First, following egress from the thymus, human MAIT cells undergo post-natal expansion and transition from a naïve-like to memory-like phenotype, potentially driven by commensal-derived antigens (Constantinides et al., 2019; Legoux et al., 2019; Legoux et al., 2020).

Second, MAIT cells appear able to mount differential clonal responses to distinct microbes in terms of TCR usage and functional capacity. Here, we will briefly discuss these emerging data, which suggest that MAIT cells are more heterogenous than previously appreciated.

The first identified activating ligands for MAIT cells were those derived from 5-amino-6-D-ribitylaminouracil (5-A-RU), a biosynthetic intermediate in the riboflavin biosynthesis pathway (Corbett et al., 2014; Kjer-Nielsen et al., 2012). It was also noted that only microbes capable of riboflavin production were able to stimulate MAIT cells, supporting the concept that MAIT cells were uniquely capable of recognizing these microbially derived antigens. The characterization of these ligands allowed for the development of MR1 tetramers loaded with 5-OP-RU (Reantragoon et al., 2013), which could stain essentially all MAIT cells, previously defined phenotypically via the co-expression of TRAV1–2, CD161, and/or CD26 (Reantragoon et al., 2013; Sharma et al., 2015). These markers were not sufficiently stringent because they are also co-expressed by T cells that do not recognize MR1, particularly in tissues or in newborn and infant blood (Ben Youssef et al., 2018). Thus, MR1–5OP-RU tetramer staining has become a cleaner, more objective criterion for defining MAIT cells. These observations, then, supported the view that MAIT cells were an innate-like T cell population of limited antigenic diversity.

Isolating MAIT cells based on either MR1–5-OP-RU tetramers or cell surface expression of TRAV1–2 and CD161 has demonstrated limited diversity in the TRAV complementary determining region (CDR) 3 region, and limited TRBV chain usage (Lepore et al., 2014; Reantragoon et al., 2013). In contrast, definition of TCR usage based on the selection of

MAIT cells on their cytokine production in response to microbial infections has revealed increasing evidence for antigenic selectivity. Accordingly, MAIT cells sorted from four donors based on the production of TNF-$\alpha$ in response to the phylogenetically diverse microbes, *Mycobacterium smegmatis*, *Candida albicans*, and *Salmonella Typhimurium* displayed pathogen selectivity through oligoclonal MAIT TCR usage (Gold et al., 2014). While in this study it was not possible to define 'microbe-specific' MAIT TCRs, there were clearly dominant responses found within an individual to a specific microbe. Notably, in response to infection with *M. smegmatis*, TNF-α was also released from TRAV1–2 negative MR1-restricted cells. One clone derived from this analysis expressed the TRAV12–2 TCR, and surprisingly responded both to microbes capable of riboflavin biosynthesis such as *M. smegmatis*, as well as to *S. pyogenes*, a microbe incapable of riboflavin production (Meermeier et al., 2016). In addition, while these studies relied on detection of TNF-α in response to microbially infected cells, it is possible that alternate functionality might be reflected via differential TCR or antigen usage. Accordingly, different combinations of cytokines can be produced by MAIT cells expressing different TRBV (Dias et al., 2017).

### 1.3 MR1T Cell Microbial Recognition

The diversity in TCR usage relative to the recognition of discrete microbes raises the possibility of presentation of antigenically diverse ligands. Cell lines expressing soluble recombinant MR1 were used to affinity purify ligands derived from *M. smegmatis* (Harriff et al., 2018). Mass spectrometry analysis revealed a quite large number of molecules associated with MR1, that could be clustered using molecular networking. To date, the molecules that could be solved and subsequently synthesized were present in the cluster that was defined based on known similarity to riboflavin-related molecules. In this report, several molecularly distinct photolumazines were identified as antigenic. Interestingly, TCR-diverse MAIT cell clones, originally derived in response to *M. tuberculosis*-infected APC, could distinguish among chemically related photolumazines. These data provided a direct demonstration of the ability of MAIT cell TCRs to confer antigenic selectivity. At present, it is not known which of the many MR1-bound ligands actually drive MAIT cell responses during different infections.

In further support of antigenic diversity, *in silico* modeling explored the potential of both microbial metabolites and pharmacologic compounds to serve as MAIT cell antigens (Salio et al., 2020). Furthermore, compounds lacking the ribityl tail of canonical MR1-restricted ligands can stimulate MAIT cells (Keller et al., 2017). It should be noted that while there is evidence for antigenic selectivity, all MAIT cells described to date are capable of binding MR1–5-OP-RU tetramers, and react to 5-OP-RU. Therefore, whether the diversity in TCR usage might reflect the selection of lower affinity, and hence more diverse TCRs in the context of different APCs or cytokine environments remains to be investigated.

With regard to how TCRs could interact with MR1-displayed ligands, the relative contribution of MR1T cell TRAV and TRBV chains is being unraveled. The canonical semi-invariant MAIT TRAV1–2 – TRAJ33/TRAJ20/TRAJ12 TCRs dock on MR1 in a conserved mode that is shifted toward the A' pocket of MR1, with the CDR3α loop optimally positioned to extend into the groove and contact the antigen (Awad et al., 2020a; Lopez-

Sagaseta et al., 2013; Patel et al., 2013). This configuration allows a germline-encoded Tyr95α from the CDR3α loop to form an 'interaction triad' with the antigen and MR1 Tyr152 (Awad et al., 2020a; Corbett et al., 2014; Eckle et al., 2014). Thus, the CDR3α loop has a crucial role in antigen recognition, with Tyr95α mediating essential hydrogen bond contacts with the ribityl chain of microbial ligands (Kjer-Nielsen et al., 2012; Lopez-Sagaseta et al., 2013). While mutagenesis of single residues within the MAIT CDR3β loop had no discernable effect on ligand recognition, exchange of the entire CDR3β region for a non-MAIT cell derived CDR3β sequence ablated antigen recognition (Reantragoon et al., 2012). However, recombinant humanized bovine MR1 exposed to ligands present in *E. coli* culture supernatant demonstrated that the MAIT CDR3β loop is also important for contact with MR1-bound antigens (Lopez-Sagaseta et al., 2013), and further studies confirmed its role in fine-tuning MAIT TCR responsiveness to these antigens (Eckle et al., 2014; Gherardin et al., 2016; Howson et al., 2018; Keller et al., 2017). More recently, four clone pairs, each having the same MAIT TRAV1–2-TRAJ33 chain, but discrete TRB chains were functionally evaluated. In this report, the TRBV was shown to allow for differential recognition of the canonical 5-OP-RU ligand, either loaded into MR1 tetramers for staining or offered in complex with plastic-bound MR1 monomers as stimulator, in alternative to APCs (Narayanan et al.). In this report, it was not demonstrated that these TCRs could uniquely recognize discrete microbes.

Concerning the recognition of MR1-presented microbial ligands by non-canonical TRAV1–2-negative MR1T TCRs, less information is available. One 5-OP-RU-reactive TRAV36 / TRBV28 TCR adopted a more central docking on MR1 as compared to invariant TRAV1–2 MAIT TCRs, with 5-OP-RU antigen specificity mediated by the germline-encoded CDR1a loop (Gherardin et al., 2016). More recently, the mode of binding of a TRAV12–2 / TRBV29–1 TCR (Meermeier et al., 2016) to MR1–5-OP-RU was unraveled. Here, the TRBV29–1β-chain interreacted with the F′-pocket of MR1 as the CDR3β loop surrounded and projected into the F′-pocket. The CDR3β loop was proximal to the MR1 A′-pocket and allowed for direct contact with the 5-OP-RU antigen. These data, demonstrate that a diverse MR1-reactive T cell repertoire could be explained by varied docking strategies that enable the use of divergent mechanisms to recognize antigens bound to MR1 (Awad et al., 2020b).

Ultimately, the importance of MR1 displaying discrete ligands, and the ability of TCRs to distinguish between these ligands could rest in the capacity of these antigens to discern microbes and so to shape the MR1T TCR repertoire akin to conventional "memory" T cells. This would require the demonstration of durable alterations in the TCR repertoire following infection or vaccination. At present, evidence here is limited, but recent work has begun to tackle this key question. Using a controlled infection of humans with live *Salmonella enterica* serovar Paratyphi A, TCR usage and functional avidity of MAIT cells following vaccination was investigated. MAIT cells with selective TRBV usage expanded upon infection and the TCR associated with this expansion displayed enhanced reactivity to antigens, suggesting selection of TCR clonotypes with greater functional avidity (Howson et al., 2018). As this report only investigated a short period after immunization (28 days), whether expanded clonotypes are persistent and associated to eventual protection remains to be determined.

## 2. Folate reactive MR1T cells

The landscape of MR1T cells was recently extended by the discovery of T cells able to recognize ligands not related to riboflavin biosynthesis. MR1 tetramers identified a rare population of T cells reactive to the folate-derivatives within circulating cells of healthy individuals (Gherardin et al., 2016). These T cells lacked TRAV1–2 gene expression, typical of MAIT cells and displayed diverse TCR usage; in addition, they were stained by MR1 tetramers loaded with Acetyl-6-Formyl-Pterin (AC-6-FP), a photodegradation product of folic acid, while failed to bind MR1–5-OP-RU tetramers. Phenotypically, these T cells predominantly expressed CD8α, displayed little or no CD161, and largely lacked expression of CD45RO, a marker of memory T cells (Lanzavecchia and Sallusto, 2000). In addition, they did not express the transcription factor PLZF, which is typical of MAIT cells. Therefore, while these T cells expressed TCRs that bound MR1, their other features were reminiscent of conventional peptide-specific rather than innate-like T cells (Fergusson et al., 2014; Lepore et al., 2018). A cell line expressing one of these TCRs failed to recognize MR1-expressing cells loaded with 5-OP-RU, but could respond to Ac-6-FP and to the closely related molecules 6-formyl-pterin (6-FP), thus confirming lack of recognition of the microbial MR1 ligands that stimulate MAIT cells. Ac-6-FP and 6-FP and are generated following photodegradation of folic acid and their physiological presence within living organisms is uncertain. Consequently, it is unclear if this recognition represents cross-reactivity of TCRs reactive to alternative sets of microbial or self MR1 ligands.

TRAV1–2- negative and PLZF-deficient MR1T cells were also described that could bind to MR1-tetramers loaded with both riboflavin-related microbial antigens and folate-derivatives (Gherardin et al., 2016). In addition, TCRs isolated from these cells displayed autoreactivity toward MR1-expressing cells. It is of note that autoreactivity has also been observed for a number of canonical MAIT TCRs reactive to 5-OP-RU. All these data suggested that varying degrees of cross-reactivity can exist among MR1T cells. Structural analysis of two distinct TRAV1–2+ and TRAV1–2- TCRs attributed their promiscuity to the flexibility of the CDR3α loop. Specifically, an alternative docking mode as compared to canonical MAIT TCRs was observed, that was shifted to the F’ pocket, and reflected interactions with MR1, the antigen and other CDR loops (Gherardin et al., 2016). Thus, different TCR modes of binding can permit MR1-recognition, and structural flexibility of the CDRs can allow recognition of multiple antigens by a single TCR. The physiological relevance of this cross-reactivity and its potential contribution to pathological reactions remain to be investigated.

## 3. Endogenous antigen reactive MR1T cells

The MR1T cell family was further enlarged by studies reporting a new T cell population capable of recognizing MR1-expressing cells in the absence of exogenous antigens (Crowther et al., 2020; Lepore et al., 2017). These MR1T cells did not react to microbial-derived molecules, nor to folate-derivatives. Instead, their activation was prevented when known MR1 antigens were exogenously provided, suggesting that these ligands blocked recognition by competing with stimulatory self-antigens for MR1 binding. Analysis of multiple circulating T cell clones from healthy individuals with these novel recognition properties revealed a diverse TCR repertoire and cell surface phenotype as well as functional

heterogeneity. Some of these MR1T cell clones preferentially responded to tumor cells, while others were also capable of recognizing monocyte-derived dendritic cells (Lepore et al., 2017), thus suggesting distinct antigen-specificities. MR1-tetramers loaded with newly identified, though yet undisclosed, molecules were shown to specifically stain some of the clones, as discussed by Chancellor and De Libero at the EMBO CD1-MR1 Conference at Oxford (unpublished), further supporting the existence of additional MR1-presented T cell ligands. The identity and variety of these antigens remains a major unsolved question in the field.

A striking feature of these T cells is the clonal diversity in terms of both target cell recognition patterns and functional properties. Individual clones display MR1-dependent recognition of tumor cells derived from different tissues, and lack reactivity to untransformed cells (Crowther et al., 2020; Lepore et al., 2017). Also, they have cytolytic activity and release pro-inflammatory mediators such as IFN-γ and TNF-α. Interestingly, some of these clones react to a broad panel of tumor cells, while others recognize a more limited set of transformed cells. These data would argue for a role in anti-tumor immunity and suggest that distinct antigens associated to different cancers might be targets of MR1-restricted T cells. It is tempting to speculate that these MR1T cells represent surveyors of cell integrity, and are able to monitor metabolic pathways whose dysregulation might lead to cancer transformation.

Recognition of normal cells by autoreactive MR1T cell clones has also been shown (Lepore et al., 2017). For example, *in vitro* experiments demonstrated MR1-dependent recognition of monocyte-derived dendritic cells (DC), which surprisingly did not result in DC killing, but rather promoted their maturation toward a licensed phenotype, instrumental for efficient priming and stimulation of T cells. In addition, a T cell clone interacted with goblet-like intestinal cells (Lepore et al., 2017) and induced transcription of the MUC2 gene, that contributes to the homeostasis of the intestinal epithelial barrier(Allaire et al., 2018; Constantinides et al., 2019; Leng et al., 2019). The analysis of clonal functional responses revealed a marked heterogeneity, with individual clones displaying T-helper 1-, T-helper-2- or T-helper 17-like cytokine secretion profiles. Some activated clones also released atypical molecules for T cells, such as the growth factors VEGF and PDGF.

In conclusion, self-reactive MR1T cells, seem to be a heterogenous population with multiple potential functions in immunity. We envisage that the identification of their recognized antigens will unveil the immunology of those T cells in physiologic and pathologic settings, and potentially support immunotherapy approaches for treatment of cancer and other diseases.

## 4. How did we get here: Aspects of Human MR1T cell Development Relevant to Antigenic Recognition

In the human adult circulation, the population of MR1T cells is largely comprised of MAIT cells. The determinants of MAIT cell dominance are not known, but recent observations from developmental studies provide considerable insight. By defining MAIT cells as TRAV1–2$^{+}$ and/or MR1 tetramer-5-OP-RU$^{+}$ it has been reported that mature MAIT cells

represent a relatively rare population in neonates as compared to adults, suggesting that they undergo a robust post-natal expansion and maturation. (Dusseaux et al., 2011; Koay et al., 2016; Martin et al., 2009; Swarbrick et al., 2020). Interestingly, the post-natal increase in the frequency of functionally mature MR1–5-OP-RU tetramer$^{+}$ MR1T cells was recently shown to overlap with the transition from a predominantly TRAV1–2$^{-}$ population in neonates, to a predominantly TRAV1–2$^{+}$ population thereafter, which was evident early (10 weeks) after birth (Swarbrick et al., 2020). Thus, human 5-OP-RU-reactive MR1T cells seem to express a more heterogenous TCR repertoire at birth, which postnatally converges into TRAV-1–2+ canonical MAIT cells. It is of note that this convergence coincides with the transition from limited microbial exposure in the womb to rapid colonization of the gut microbiome. In mice, MAIT cell expansion is linked to the acquisition of the intestinal microbiome (Constantinides et al., 2019; Legoux et al., 2019; Legoux et al., 2020). Therefore, it is likely that microbial ligands drive preferential expansion TRAV1–2$^{+}$ MAIT cells during development in humans.

## 5. Conclusions

Advances in structural biology, cellular immunology, and most notably the development of novel tools such as MR1 tetramers, has allowed for rapid expansion of knowledge of this growing family of phenotypically and functionally diverse MR1T cells. As summarized above, MR1 can present a diverse array of ligands, and these in turn can lead to expansion of diverse MR1T cell subsets. The context of these expansions, such as tissue residence, cytokine milieu and antigen presentation capacity of various APCs remains poorly understood. Clearly, further investigation may unravel crucial aspects of the immunological role of MR1T cells and enable their immunotherapeutic use for the prevention and treatment of microbial infection, cancer, and autoimmune disease.

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## Abbreviations:

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| **TCR** | T cell receptor |
| **CDR** | complementary determining region |
| **TRAV** | T cell receptor alpha variable |
| **TRBV** | T cell receptor beta variable |
| **TNF-α** | tumor necrosis factor alpha |
| **IFN-γ** | Interferon gamma |

## References


Allaire JM, Morampudi V, Crowley SM, Stahl M, Yu H, Bhullar K, Knodler LA, Bressler B, Jacobson K, Vallance BA, 2018 Frontline defenders: goblet cell mediators dictate host-microbe interactions in the intestinal tract during health and disease. Am J Physiol Gastrointest Liver Physiol 314, G360–G377. [PubMed: 29122749]

Awad W, Ler GJM, Xu W, Keller AN, Mak JYW, Lim XY, Liu L, Eckle SBG, Le Nours J, McCluskey J, Corbett AJ, Fairlie DP, Rossjohn J, 2020a The molecular basis underpinning the potency and specificity of MAIT cell antigens. Nat Immunol 21, 400–411. [PubMed: 32123373]

Awad W, Meermeier EW, Sandoval-Romero ML, Le Nours J, Worley AH, Null MD, Liu L, McCluskey J, Fairlie DP, Lewinsohn DM, Rossjohn J, 2020b Atypical TRAV1–2- T cell receptor recognition of the antigen-presenting molecule MR1. J Biol Chem.

Ben Youssef G, Tourret M, Salou M, Ghazarian L, Houdouin V, Mondot S, Mburu Y, Lambert M, Azarnoush S, Diana JS, Virlouvet AL, Peuchmaur M, Schmitz T, Dalle JH, Lantz O, Biran V, Caillat-Zucman S, 2018 Ontogeny of human mucosal-associated invariant T cells and related T cell subsets. J Exp Med 215, 459–479. [PubMed: 29339446]

Constantinides MG, Link VM, Tamoutounour S, Wong AC, Perez-Chaparro PJ, Han SJ, Chen YE, Li K, Farhat S, Weckel A, Krishnamurthy SR, Vujkovic-Cvijin I, Linehan JL, Bouladoux N, Merrill ED, Roy S, Cua DJ, Adams EJ, Bhandoola A, Scharschmidt TC, Aube J, Fischbach MA, Belkaid Y, 2019 MAIT cells are imprinted by the microbiota in early life and promote tissue repair. Science 366.

Corbett AJ, Eckle SB, Birkinshaw RW, Liu L, Patel O, Mahony J, Chen Z, Reantragoon R, Meehan B, Cao H, Williamson NA, Strugnell RA, Van Sinderen D, Mak JY, Fairlie DP, Kjer-Nielsen L, Rossjohn J, McCluskey J, 2014 T-cell activation by transitory neo-antigens derived from distinct microbial pathways. Nature 509, 361–365. [PubMed: 24695216]

Crowther MD, Dolton G, Legut M, Caillaud ME, Lloyd A, Attaf M, Galloway SAE, Rius C, Farrell CP, Szomolay B, Ager A, Parker AL, Fuller A, Donia M, McCluskey J, Rossjohn J, Svane IM, Phillips JD, Sewell AK, 2020 Genome-wide CRISPR-Cas9 screening reveals ubiquitous T cell cancer targeting via the monomorphic MHC class I-related protein MR1. Nat Immunol 21, 178–185. [PubMed: 31959982]

Dias J, Leeansyah E, Sandberg JK, 2017 Multiple layers of heterogeneity and subset diversity in human MAIT cell responses to distinct microorganisms and to innate cytokines. Proc Natl Acad Sci U S A 114, E5434–E5443. [PubMed: 28630305]

Dusseaux M, Martin E, Serriari N, Peguillet I, Premel V, Louis D, Milder M, Le Bourhis L, Soudais C, Treiner E, Lantz O, 2011 Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting T cells. Blood 117, 1250–1259. [PubMed: 21084709]

Eckle SB, Birkinshaw RW, Kostenko L, Corbett AJ, McWilliam HE, Reantragoon R, Chen Z, Gherardin NA, Beddoe T, Liu L, Patel O, Meehan B, Fairlie DP, Villadangos JA, Godfrey DI, Kjer-Nielsen L, McCluskey J, Rossjohn J, 2014 A molecular basis underpinning the T cell receptor heterogeneity of mucosal-associated invariant T cells. J Exp Med 211, 1585–1600. [PubMed: 25049336]

Fergusson JR, Smith KE, Fleming VM, Rajoriya N, Newell EW, Simmons R, Marchi E, Bjorkander S, Kang YH, Swadling L, Kurioka A, Sahgal N, Lockstone H, Baban D, Freeman GJ, Sverremark-Ekstrom E, Davis MM, Davenport MP, Venturi V, Ussher JE, Willberg CB, Klenerman P, 2014 CD161 defines a transcriptional and functional phenotype across distinct human T cell lineages. Cell Rep 9, 1075–1088. [PubMed: 25437561]

Gherardin NA, Keller AN, Woolley RE, Le Nours J, Ritchie DS, Neeson PJ, Birkinshaw RW, Eckle SB, Waddington JN, Liu L, Fairlie DP, Uldrich AP, Pellicci DG, McCluskey J, Godfrey DI, Rossjohn J, 2016 Diversity of T Cells Restricted by the MHC Class I-Related Molecule MR1 Facilitates Differential Antigen Recognition. Immunity 44, 32–45. [PubMed: 26795251]

Godfrey DI, Koay HF, McCluskey J, Gherardin NA, 2019 The biology and functional importance of MAIT cells. Nat Immunol 20, 1110–1128. [PubMed: 31406380]

Gold MC, McLaren JE, Reistetter JA, Smyk-Pearson S, Ladell K, Swarbrick GM, Yu YY, Hansen TH, Lund O, Nielsen M, Gerritsen B, Kesmir C, Miles JJ, Lewinsohn DA, Price DA, Lewinsohn DM,

2014 MR1-restricted MAIT cells display ligand discrimination and pathogen selectivity through distinct T cell receptor usage. J Exp Med 211, 1601–1610. [PubMed: 25049333]

Harriff MJ, McMurtrey C, Froyd CA, Jin H, Cansler M, Null M, Worley A, Meermeier EW, Swarbrick G, Nilsen A, Lewinsohn DA, Hildebrand W, Adams EJ, Lewinsohn DM, 2018 MR1 displays the microbial metabolome driving selective MR1-restricted T cell receptor usage. Sci Immunol 3.

Howson LJ, Napolitani G, Shepherd D, Ghadbane H, Kurupati P, Preciado-Llanes L, Rei M, Dobinson HC, Gibani MM, Teng KWW, Newell EW, Veerapen N, Besra GS, Pollard AJ, Cerundolo V, 2018 MAIT cell clonal expansion and TCR repertoire shaping in human volunteers challenged with Salmonella Paratyphi A. Nature communications 9, 253.

Keller AN, Eckle SB, Xu W, Liu L, Hughes VA, Mak JY, Meehan BS, Pediongco T, Birkinshaw RW, Chen Z, Wang H, D'Souza C, Kjer-Nielsen L, Gherardin NA, Godfrey DI, Kostenko L, Corbett AJ, Purcell AW, Fairlie DP, McCluskey J, Rossjohn J, 2017 Drugs and drug-like molecules can modulate the function of mucosal-associated invariant T cells. Nat Immunol 18, 402–411. [PubMed: 28166217]

Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, Liu L, Bhati M, Chen Z, Kostenko L, Reantragoon R, Williamson NA, Purcell AW, Dudek NL, McConville MJ, O'Hair RA, Khairallah GN, Godfrey DI, Fairlie DP, Rossjohn J, McCluskey J, 2012 MR1 presents microbial vitamin B metabolites to MAIT cells. Nature 491, 717–723. [PubMed: 23051753]

Koay HF, Gherardin NA, Enders A, Loh L, Mackay LK, Almeida CF, Russ BE, Nold-Petry CA, Nold MF, Bedoui S, Chen Z, Corbett AJ, Eckle SB, Meehan B, d'Udekem Y, Konstantinov IE, Lappas M, Liu L, Goodnow CC, Fairlie DP, Rossjohn J, Chong MM, Kedzierska K, Berzins SP, Belz GT, McCluskey J, Uldrich AP, Godfrey DI, Pellicci DG, 2016 A three-stage intrathymic development pathway for the mucosal-associated invariant T cell lineage. Nat Immunol 17, 1300–1311. [PubMed: 27668799]

Lanzavecchia A, Sallusto F, 2000 Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells. Science 290, 92–97. [PubMed: 11021806]

Legoux F, Bellet D, Daviaud C, El Morr Y, Darbois A, Niort K, Procopio E, Salou M, Gilet J, Ryffel B, Balvay A, Foussier A, Sarkis M, El Marjou A, Schmidt F, Rabot S, Lantz O, 2019 Microbial metabolites control the thymic development of mucosal-associated invariant T cells. Science 366, 494–499. [PubMed: 31467190]

Legoux F, Salou M, Lantz O, 2020 MAIT Cell Development and Functions: the Microbial Connection. Immunity 53, 710–723. [PubMed: 33053329]

Leng T, Akther HD, Hackstein CP, Powell K, King T, Friedrich M, Christoforidou Z, McCuaig S, Neyazi M, Arancibia-Carcamo CV, Hagel J, Powrie F, Oxford IBDI, Peres RS, Millar V, Ebner D, Lamichhane R, Ussher J, Hinks TSC, Marchi E, Willberg C, Klenerman P, 2019 TCR and Inflammatory Signals Tune Human MAIT Cells to Exert Specific Tissue Repair and Effector Functions. Cell Rep 28, 3077–3091 e3075. [PubMed: 31533032]

Lepore M, Kalinicenko A, Colone A, Paleja B, Singhal A, Tschumi A, Lee B, Poidinger M, Zolezzi F, Quagliata L, Sander P, Newell E, Bertoletti A, Terracciano L, De Libero G, Mori L, 2014 Parallel T-cell cloning and deep sequencing of human MAIT cells reveal stable oligoclonal TCRbeta repertoire. Nature communications 5, 3866.

Lepore M, Kalinichenko A, Calogero S, Kumar P, Paleja B, Schmaler M, Narang V, Zolezzi F, Poidinger M, Mori L, De Libero G, 2017 Functionally diverse human T cells recognize non-microbial antigens presented by MR1. Elife 6.

Lepore M, Mori L, De Libero G, 2018 The Conventional Nature of Non-MHC-Restricted T Cells. Frontiers in immunology 9, 1365. [PubMed: 29963057]

Lopez-Sagaseta J, Dulberger CL, Crooks JE, Parks CD, Luoma AM, McFedries A, Van Rhijn I, Saghatelian A, Adams EJ, 2013 The molecular basis for Mucosal-Associated Invariant T cell recognition of MR1 proteins. Proc Natl Acad Sci U S A 110, E1771–1778. [PubMed: 23613577]

Martin E, Treiner E, Duban L, Guerri L, Laude H, Toly C, Premel V, Devys A, Moura IC, Tilloy F, Cherif S, Vera G, Latour S, Soudais C, Lantz O, 2009 Stepwise development of MAIT cells in mouse and human. PLoS Biol 7, e54. [PubMed: 19278296]

Meermeier EW, Laugel BF, Sewell AK, Corbett AJ, Rossjohn J, McCluskey J, Harriff MJ, Franks T, Gold MC, Lewinsohn DM, 2016 Human TRAV1–2-negative MR1-restricted T cells detect S. pyogenes and alternatives to MAIT riboflavin-based antigens. Nature communications 7, 12506.

Narayanan GA, McLaren JE, Meermeier EW, Ladell K, Swarbrick GM, Price DA, Tran JG, Worley AH, Vogt T, Wong EB, Lewinsohn DM, The MAIT TCRβ chain contributes to discrimination of microbial ligand. Immunology & Cell Biology n/a.

Patel O, Kjer-Nielsen L, Le Nours J, Eckle SB, Birkinshaw R, Beddoe T, Corbett AJ, Liu L, Miles JJ, Meehan B, Reantragoon R, Sandoval-Romero ML, Sullivan LC, Brooks AG, Chen Z, Fairlie DP, McCluskey J, Rossjohn J, 2013 Recognition of vitamin B metabolites by mucosal-associated invariant T cells. Nature communications 4, 2142.

Porcelli S, Yockey CE, Brenner MB, Balk SP, 1993 Analysis of T cell antigen receptor (TCR) expression by human peripheral blood CD4–8- alpha/beta T cells demonstrates preferential use of several V beta genes and an invariant TCR alpha chain. J Exp Med 178, 1–16. [PubMed: 8391057]

Reantragoon R, Corbett AJ, Sakala IG, Gherardin NA, Furness JB, Chen Z, Eckle SB, Uldrich AP, Birkinshaw RW, Patel O, Kostenko L, Meehan B, Kedzierska K, Liu L, Fairlie DP, Hansen TH, Godfrey DI, Rossjohn J, McCluskey J, Kjer-Nielsen L, 2013 Antigen-loaded MR1 tetramers define T cell receptor heterogeneity in mucosal-associated invariant T cells. J Exp Med 210, 2305–2320. [PubMed: 24101382]

Reantragoon R, Kjer-Nielsen L, Patel O, Chen Z, Illing PT, Bhati M, Kostenko L, Bharadwaj M, Meehan B, Hansen TH, Godfrey DI, Rossjohn J, McCluskey J, 2012 Structural insight into MR1-mediated recognition of the mucosal associated invariant T cell receptor. J Exp Med.

Salio M, Awad W, Veerapen N, Gonzalez-Lopez C, Kulicke C, Waithe D, Martens AWJ, Lewinsohn DM, Hobrath JV, Cox LR, Rossjohn J, Besra GS, Cerundolo V, 2020 Ligand-dependent downregulation of MR1 cell surface expression. Proc Natl Acad Sci U S A 117, 10465–10475. [PubMed: 32341160]

Sharma PK, Wong EB, Napier RJ, Bishai WR, Ndung'u T, Kasprowicz VO, Lewinsohn DA, Lewinsohn DM, Gold MC, 2015 High expression of CD26 accurately identifies human bacteria-reactive MR1-restricted MAIT cells. Immunology 145, 443–453. [PubMed: 25752900]

Swarbrick GM, Gela A, Cansler ME, Null MD, Duncan RB, Nemes E, Shey M, Nsereko M, Mayanja-Kizza H, Kiguli S, Koh J, Hanekom WA, Hatherill M, Lancioni C, Lewinsohn DM, Scriba TJ, Lewinsohn DA, 2020 Postnatal Expansion, Maturation, and Functionality of MR1T Cells in Humans %U https://www.frontiersin.org/article/10.3389/fimmu.2020.556695. Frontiers in immunology 11, %7 %8 2020-September-2016 %2029 Original Research %# %! Human MR2021 T cell development %* %<.

Tilloy F, Treiner E, Park SH, Garcia C, Lemonnier F, de la Salle H, Bendelac A, Bonneville M, Lantz O, 1999 An invariant T cell receptor alpha chain defines a novel TAP-independent major histocompatibility complex class Ib-restricted alpha/beta T cell subpopulation in mammals. J Exp Med 189, 1907–1921. [PubMed: 10377186]

Treiner E, Duban L, Bahram S, Radosavljevic M, Wanner V, Tilloy F, Affaticati P, Gilfillan S, Lantz O, 2003 Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. Nature 422, 164–169. [PubMed: 12634786]

## Highlights

- MR1 can present multiple distinct ligands to T cells
- Diverse Human MR1 restricted T cells recognise distinct microbial or self/tumour antigens
- Individual MR1-restricted TCR display ligand discriminations
- Some MR1-restricted TCRs are cross-reactive toward multiple ligands
- Human MR1-restricted T cells are functionally heterogeneous

**Table 1:**

The Expanding Universe of Known MR1-Restricted T (MR1T) Cells

| Name | TCR Alpha Usage | Tetramer Staining | | Cell Surface Phenotype | Transcription factors | Antigen Recognition | Effector Function | References |
|---|---|---|---|---|---|---|---|---|
| | | 5-OP-RU | 6-FP | | | | | |
| Mucosal Associated Invariant T (MAIT) Cells | TRAV1–2-TRAJ33/20/12 | Yes | A limited proportion of these cells | CD161++ CD26+ | PLZF RORγT | Microbes Intracellular Extracellular Riboflavin metabolites Photolumazines Diverse, Non-ribityl containing compounds | Proinflammatory Cytokines Cytolytic Anti-microbial | Godfrey et al., 2019: Keller et al., 2017; Salio et al., 2020; Hariff et al., 2018 |
| Microbial-reactive | TRAV1–2 TRAV12–2 TRAV36 | Yes | No | CD161++ CD26+ | PLZF RORγT | Microbes Intracellular Extracellular Riboflavin metabolites Photolumazines | Proinflammatory Cytokines Cytolytic Anti-microbial | Gherardin et al., 2016; Meermeier et al., 2016; Hariff et al., 2018 Swarbrick et al., 2020 |
| | | | | | | Unknow microbial non-riboflavin related ligands | | |
| Folate-reactive | Polyclonal | No | Yes | Diverse | Diverse | 6-FP and Ac-6-FP | Unknown | Gherardin et al., 2016 |
| Self-reactive | Polyclonal | No | No | Diverse | Diverse | Tumor cells and/or Mo-DC | Cytolytic Th1-, Th2- and Th17-like Growth factors (e.g. VEGF, PDGF) | Lepore et al., 2017; Crowther et al., 2020 |