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Revealing the protective and pathogenic potential of MAIT cells

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ABSTRACT

Mucosal-associated Invariant T (MAIT) cells represent a large proportion of T cells in human blood, and are also present throughout the body, being concentrated at mucosal sites. Their high level of conservation throughout mammalian evolution and recognition of conserved microbial antigens, derived from precursors of riboflavin (vitamin B2) biosynthesis, suggest an important role in protective immunity to pathogens. However, the picture that is emerging of MAIT cell immune function is increasingly complex, with numerous correlations of MAIT cell numbers with human diseases, and with recent studies demonstrating their pathogenic potential. The conditions that drive MAIT cell responses towards a protective versus pathogenic role are only beginning to be deciphered and, yet, must be understood for any attempt to harness MAIT cells therapeutically. In this review we summarise our current knowledge of immune protection and pathology driven by MAIT cells, models used to study their role in immunity and steps towards elucidating the immune signals driving these responses.

1. MAIT cell recognise a new class of antigens

MAIT cells are a T cell subset present in most mammals. In humans, they are highly abundant in the blood and mucosal tissues, particularly at sites of pathogen entry, including the lungs, liver, intestines and genitourinary tract, representing an average of 5% of T cells in blood and up to 50% of T cells in liver (Dusseaux et al., 2011; Le Bourhis et al., 2010; Martin et al., 2009; Tang et al., 2013). MAIT cells express a semi-invariant αβ T cell antigen receptor (TCR), which is restricted to the monomorphic Major Histocompatibility Complex (MHC) related protein-1 (MR1) (Le Bourhis et al., 2011; Tilloy et al., 1999). In humans, this TCR comprises TRAV1-TRAJ33 (Vα7.2-Jα33/20/12) TCR α-chain, preferentially assembled with TRBV20$^+$ (Vβ2) or TRBV6$^+$ (Vβ13) TCR β-chains, and in mice TRAV1-TRAJ33 (Vα19-Jα33) is mostly paired with TRBV19 (Vβ6) or TRBV13 (Vβ8) β-chains (Reantragoon et al., 2013; Tilloy et al., 1999). Although initially described as a conserved CD4$^-$CD8$^-$ double negative (DN) T cell population in human blood (Porcelli et al., 1993), MAIT cells can be CD8$^+$, CD4$^+$ or CD4$^-$CD8$^-$ (double negative, DN), with CD8$^+$ and DN cells making the majority of the cells in humans and mice, and a small number of CD4$^+$CD8$^+$ double positive cells also present (Kurioka et al., 2017; Martin et al., 2009; Rahimpour et al., 2015; Reantragoon et al., 2013). They also express markers typical of natural killer (NK) cells, including CD161 (in human), and CD26 (Sharma et al., 2015), and are described as having an "effector/memory" phenotype, being CD44^{hi} and CD62L^{lo} (Dusseaux et al., 2011; Le Bourhis et al., 2010; Martin et al., 2009). A recent phenotypic analysis of human blood MAIT cells, including their changes with age was performed, using MR1 tetramers to accurately identify MAIT cells (Gherardin et al., 2018b), further adding to our understanding of these cells.

In 2003, Treiner et al. demonstrated the requirement for commensal microbiota and MR1 for MAIT cell development (Treiner et al., 2003). However, it was not until two key papers (Gold et al., 2010; Le Bourhis et al., 2010) were published in 2010 describing a range of microbes, both bacteria and yeast, that activated, or did not activate MAIT cells, that the identity of MAIT cell antigens could begin to be unravelled, and a new class of antigen could be defined. This story of how this discovery unfolded is described in detail elsewhere (Kjer-Nielsen et al., 2018). The MR1-bound antigens that activate MAIT cells are small ring compounds (Corbett et al., 2014; Kjer-Nielsen et al., 2012), and thus quite distinct from other T cell antigens, which are either peptide or lipid-based molecules. These antigens, the most potent of which are 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) and 5-(2-oxoethylideneamino)-6-D-ribitylaminouracil (5-OE-RU), are formed from a microbial riboflavin (vitamin B2) synthesis precursor, 5-amino-6-D-ribitylaminouracil (5-A-RU), and small carbon metabolites methylglyoxal and glyoxal, respectively (Corbett et al., 2014). Additionally, folate (vitamin B9)-based MR1 ligands, including 6-Formyl pterin (6-FP), have been described (Kjer-Nielsen et al., 2012). These are non-stimulatory for MAIT cells and can competitively inhibit their response (Eckle et al., 2014; Keller et al., 2017; Patel et al., 2013; Soudais et al., 2015), but the physiological relevance of these molecules is currently

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unclear.

More recently, small populations of MR1-reactive T cells have been described, which recognise folate-derived antigens ("atypical MAIT cells" (Gherardin et al., 2016)), or which are diverse TCR-bearing cells more akin to conventional αβ-T cells ("MR1T cells" (Lepore et al., 2017)). Other studies suggest that diversity within the MAIT TCR repertoire may lead to differential recognition of pathogens (Dias et al., 2017; Gold et al., 2014; Howson et al., 2018; Lepore et al., 2014; Reantragoon et al., 2013). Additionally, the search for potent microbial ligands other than the riboflavin-based antigens is ongoing in the field. However, for the purposes of this review, we will concentrate on the large majority of MAIT cells, which are MR1-5-OP-RU reactive.

2. Tools to study the MAIT cell responses in mice

In humans, MAIT cells have been defined variously in the past as $CD8^+$ or DN T cells, or with combinations of markers including TRAV1-2 (Ab 3C10 (Martin et al., 2009)) and CD161 (in humans), CD26, IL-18R and the promyelocytic leukemia zinc finger (PLZF) transcription factor (Gold et al., 2010; Martin et al., 2009; Sharma et al., 2015). However, to study physiological immune responses to pathogens, whole animal models are indispensable tools, but until recently the analysis of MAIT cells in mice was limited by the lack of reagents to specifically detect mouse MAIT cells. Additionally, in contrast to humans where MAIT cells are present in relatively high numbers, in SPF-housed mice they comprise a much smaller proportion of T cells (e.g. ≤ 0.1% of blood T cells) (Chen et al., 2017; Rahimpour et al., 2015). Thus, while in vitro analysis of MAIT cells has been possible using cell lines, or human cells from a range of patients or healthy donors, studying MAIT cells in vivo experimentally, such as during infection, has been more challenging. Several tools and approaches have been developed to circumvent this problem.

The identification of MR1-bound ligands, including 6-FP (non-stimulatory) as small vitamin-based compounds (Kjer-Nielsen et al., 2012) and elucidation of the formation of 5-OP-RU from the riboflavin precursor 5-A-RU, and small carbon metabolite methylglyoxal (Corbett et al., 2014), allowed the subsequent development of MR1-Ag-tetramers (Corbett et al., 2014; Reantragoon et al., 2013) now available through the NIH Core Tetramer Facility. These allow definitive identification of even small numbers of MAIT cells in a range of mammalian species. In humans MAIT cells detected using MR1-teramers are almost completely overlapping with the previous definition of $CD3^+TRAV1\text{-}2^+CD161^{++}$ cells (Corbett et al., 2014; Reantragoon et al., 2013). Using mouse MR1-tetramers, MAIT cell can now be detected in a range of tissues, including lungs, liver, spleen, thymus, intestinal lamina propria, lymph nodes, female reproductive tract, and stomach where they represent only a small proportion of T cells in inbred strains such as C57BL/6 and BALB/c (Chen et al., 2017; D'Souza et al., 2018; Koay et al., 2016; Rahimpour et al., 2015).

In germ-free mice, MAIT cells are virtually undetectable in the periphery (Koay et al., 2016; Treiner et al., 2003). The addition of even single strains of bacteria was found to restore the frequency of MAIT cells (Le Bourhis et al., 2010), indicating that their development was microbe dependent. $MR1^{-/-}$ mice lack detectable MAIT cells (Treiner et al., 2003) and have been used in several studies to define a role for MAIT cells in immune protection against bacterial infections (Chua and Hansen, 2010; D'Souza et al., 2018; Georgel et al., 2011; Meierovics et al., 2013) and in inflammatory conditions, including experimental autoimmune encephalomyelitis (Croxford et al., 2006), and recently graft versus host disease (Varelias et al., 2018). These mice have also been crossed to DBA/1 J mice for use in the collagen induced arthritis model (Chiba et al., 2012) and to non-obese diabetic (NOD) mice to assess the role of MAIT cells in type 1 diabetes (Rouxel et al., 2017).

To assess the function and response of MAIT cells in vivo, TCR transgenic mice expressing the Vα19Jα33 (Vα19*i*) TCR alpha chain have been generated by a few groups and have also been crossed to $MR1^{-/-}$ mice (Kawachi et al., 2006; Martin et al., 2009; Treiner et al., 2003). These mice have been used in many studies, significantly advancing the field. However, recently, in part due to the development of MR1-5-OP-RU tetramers, differences in the phenotype of wild type and transgenic MAIT cells have been revealed. MAIT cells from $Vα19iCα^{-/-}$ mice have higher TCR expression than those from wild-type mice (Rahimpour et al., 2015). MAIT cells in Vα19 TCR Tg mice also differ from those in wild-type mice, and from human MAIT cells, as they lack PLZF expression and have a naïve $CD44^{lo}$ phenotype (Martin et al., 2009; Rahimpour et al., 2015). Additionally, a proportion of MR1-5-OP-RU tetramer-reactive "MAIT-like" cells in transgenic mice develop in an MR1-independent manner, suggesting they are restricted by MHC molecules during thymic development (Reantragoon et al., 2013; Sakala et al., 2015). Thus, although a valuable tool, caution should be used when interpreting data generated with MAIT TCR transgenic mice.

Additional mouse strains have been used by various groups to assess MAIT cell function. The CAST/EiJ mouse, a wild-derived strain, has 20 times more MAIT cells than the C57BL/6 mouse due to a difference in the TCRα locus (Cui et al., 2015). This strain has been utilised by the Lantz group, who also made use of the expression by MAIT cells of the retinoid-related orphan receptor (ROR)γt (Chen et al., 2017; Rahimpour et al., 2015), developing a congenic strain crossed to a RORγt GFP reporter, allowing $RORγt^+$ MAIT cells to be tracked, and revealing a protective role in *E. coli* bladder infection (Cui et al., 2015).

Wakao et al. sought to more closely model human MAIT cells in mice by generating "reMAIT cells" (induced pluripotent stem cells derived from MAIT cells), and demonstrated successful reconstitution of NOD/SCID or NOD/Shi-scid $IL2Rγ^{-/-}$ mice with these cells (Wakao et al., 2013). This, or similar humanised mouse models, may prove useful for the future development of MAIT cell therapy. Their rapid effector response and restriction to the monomorphic MR1 molecule may give MAIT cells an advantage over conventional T cells, and supplementation of patients in a number of conditions where MAIT cells are depleted (discussed below) may help recover immune competence (Wakao et al., 2017).

Recently, efforts have been made to boost MAIT cells in wild-type C57BL/6 mice with the dual aims of understanding the signals driving their activation, and increasing their numbers in order to more readily assess their immune role during infection. The intranasal infection of mice with *Salmonella enterica* sevovar Typhimurium led to a remarkable enrichment of MAIT cells in the lungs (up to ~50% of T cells) in a short time (7 days), and a similar MAIT cell boosting effect could be achieved with synthetic 5-OP-RU antigen co-administered with Toll-Like Receptor (TLR) agonists (Chen et al., 2017). These models also serve as a source of MAIT cells for adoptive transfer experiments into immune deficient mice, thus allowing genetic dissection of the mechanisms of protection. For example, immunodeficient $RAG2^{-/-}γC^{-/-}$ mice could be protected against *Legionella longbeachae* infection by adoptively transferred MAIT cells from C57BL/6 mice but not $IFNγ^{-/-}$ mice that had been primed with *Salmonella* Typhimurium (Wang et al., 2018).

3. MAIT cell response and evidence for a protective role in bacterial infection

MAIT cells have been shown to respond rapidly to bacteria by production of cytokines, including IFNγ, TNF, GM-CSF and IL-17 (Gold et al., 2010; Le Bourhis et al., 2010), consistent with their expression of transcription factors including T-bet and RORγt (Chen et al., 2017; Dusseaux et al., 2011; Koay et al., 2016; Martin et al., 2009). They also are capable of direct cytotoxicity of infected cells, through production of perforin and granzyme B, which are induced upon activation (Kurioka et al., 2015; Zinser et al., 2018), and may modulate antibody production (Bennett et al., 2017). A key concept in MAIT cell immune recognition of microbial antigens is that the riboflavin biosynthesis pathway is an essential metabolic pathway for many bacteria and yeast, but absent in mammals. Thus the MAIT cell antigens act as a pathogen