

1 Understanding and modulating the MR1 metabolite antigen  
2 presentation pathway

3  
4 Hamish E.G. McWilliam<sup>1,2</sup> and Mariolina Salio<sup>3</sup>

5  
6 <sup>1</sup> Department of Microbiology and Immunology, The University of Melbourne, The Peter  
7 Doherty Institute for Infection and Immunity, Melbourne, VIC, 3000, Australia

8 <sup>2</sup> Department of Biochemistry and Molecular Biology, The University of Melbourne, Bio21  
9 Molecular Science and Biotechnology Institute, Parkville, VIC 3010, Australia

10 <sup>3</sup> Medical Research Council Human Immunology Unit, Medical Research Council Weatherall  
11 Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford,  
12 Oxford, OX3 9DS, UK

13  
14  
15 Correspondence: [hamish.mcwilliam@unimelb.edu.au](mailto:hamish.mcwilliam@unimelb.edu.au) or [mariolina.salio@imm.ox.ac.uk](mailto:mariolina.salio@imm.ox.ac.uk)

## Abstract

The MHC class I-related protein, MR1, presents small metabolite antigens to an unusual subset of innate-like T cells. Herein, we highlight recent progress in our understanding of MR1's unique antigen presenting pathway, with features of both MHC class I and class II antigen presentation, as highlighted during the EMBO Workshop: CD1-MR1, Beyond MHC-restricted lymphocytes, Oxford, 2019. There is increasing evidence for a role of MR1 restricted T cells in several immune contexts, from cancer to autoimmunity and infections, and therapeutic harnessing of this important biological axis through generation of agonist and antagonist MR1 ligands requires a thorough understanding of the molecular mechanisms of MR1-dependent antigen presentation.

## Highlights:

- MR1 presents a range of small organic ligands to modulate specific T cell subsets
- MR1 ligands are loaded in the ER, but alternative endo-lysosomal loading can occur
- MR1 ligands are generally unstable unless bound in the antigen presenting groove
- Evasion from MR1 antigen presentation can occur

Abbreviations: MHC, Major histocompatibility complex; MR1, MHC class I-related protein 1; MR1-T cells, MR1-restricted T cells; MAIT cells, mucosal associated invariant T cells; 5-OP-RU, 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil; 6-FP, 6-formyl pterin; FMN, flavin mononucleotide; FAD, flavin adenine dinucleotide; APC, antigen presenting cell.

## 1. Introduction

Major histocompatibility complex (MHC) class I-related protein 1 (MR1) is closely related to MHC class I molecules in structure (Hashimoto et al., 1995) and function, in that both are non-

covalently bound to  $\beta_2$ -microglobulin and present antigens (Ag) to activate T cells. Yet MR1 has the distinct difference that instead of binding peptide antigens, it is tailored to capture small metabolite antigens derived primarily from microbes (Corbett et al., 2014; Kjer-Nielsen et al., 2012). Once bound, MR1-metabolite complexes are displayed at the cell surface for recognition by innate-like MR1-restricted T (MR1-T) cells of which the most numerous are mucosal associated invariant T (MAIT) cells. MAIT cells express a semi-invariant  $\alpha\beta$  T cell receptor that recognises MR1 presenting riboflavin-derived microbial antigen, whereas other MR1-T cells are less abundant and express more diverse T cell receptors that recognise MR1 alone, or MR1 presenting other classes of antigens (as recently reviewed by Souter and Eckle (2020)). Compared to the almost infinite range of peptide antigens presented by MHC molecules, MR1 is known to bind a more limited number of metabolites, although the list of bona fide MR1 ligands is increasing (McWilliam and Villadangos, 2018). The foremost of these ligands are derived from transient intermediates of the synthesis of riboflavin (vitamin B<sub>2</sub>), a biosynthetic pathway which is present in a diverse range of bacteria and yeasts, but not viruses (Corbett et al., 2014; Kjer-Nielsen et al., 2012; Le Bourhis et al., 2010). Hence in capturing and presenting these metabolites, MR1 displays a unique molecular signature of microbes to alert MAIT cells of their presence. Countering such T cell agonists, are an increasing range of non-activating ligands, which, for example are derived from folic acid and do not stimulate MAIT cells and can even block activation from riboflavin-related antigen (Eckle et al., 2015; Patel et al., 2013; Soudais et al., 2015).

Activation of MAIT cells by MR1-antigen complexes elicits several immunomodulatory outcomes, including antimicrobial immunity by killing infected cells and releasing inflammatory cytokines (Dusseaux et al., 2011; Gold et al., 2010; Le Bourhis et al., 2010; Walker et al., 2012), recruiting the adaptive response (Meierovics and Cowley, 2016; Meierovics et al., 2013; Salio et al., 2017), or maintaining homeostasis through promoting

tissue repair at barrier tissues (Constantinides et al., 2019; Hinks et al., 2019; Lamichhane et al., 2019; Leng et al., 2019). Thus, the MR1-MAIT cell axis is a highly specific mode of microbial detection through their unique metabolic by-products.

Several aspects make the MR1 antigen display system a highly attractive and potentially tractable therapeutic target. Firstly, the MR1 protein is derived from a single gene, is highly conserved across mammals with limited polymorphisms in humans (Seshadri et al., 2016), and thus, any therapeutic ligands or antibodies are likely to apply to all human populations and potentially have veterinary applications. Secondly, MAIT cells are highly abundant in humans, have immunomodulatory effects on other effector cells and are positioned at barrier tissues, which are the first sites of pathogen encounter (Dusseaux et al., 2011; Leng et al., 2019). Thirdly, MR1 exhibits some structural ability to bind a range of ligands, including natural riboflavin- or folic acid-derived metabolites, modified synthetic ligands such as the stable JYM72 (Awad et al., 2020; Mak et al., 2017), or drug-like compounds such as the anti-inflammatory drug diclofenac (Keller et al., 2017). Finally, advances have been made in the discovery and synthesis of novel MR1-binding ligands, and many of these have been shown to modulate MR1 presentation and/or MAIT cell activity (Awad et al., 2020; Braganza et al., 2020; Braganza et al., 2019; Lange et al., 2020; Ler et al., 2019; Mak et al., 2017; Salio et al., 2020). In this review we focus on the current understanding of the MR1 trafficking pathway and how this informs the targeting and design of therapeutic MR1 ligands, the range of compounds that modulate MR1 presentation and MAIT cell function, and finally we outline potential applications of such therapeutic ligands.

## **2. Insights from the MR1 presentation pathway for novel ligand design**

MR1 has the fascinating ability to capture and present antigen located both inside the cell, from intracellular microbes (Le Bourhis et al., 2013), or outside the cell, from the extracellular

microbial metabolite milieu, including evidence that ligands generated at one site in the body can activate cells at different sites (Constantinides et al., 2019; Legoux et al., 2019). Classically, the presentation of these endogenous and exogenous sources of protein-derived antigen by MHC molecules is handled separately by MHC class I and II molecules, which have differing trafficking pathways and co-factors (Rock et al., 2016). Intriguingly, cellular evidence indicates that MR1 alone can achieve this feat, although the mechanisms that are still being delineated, particularly regarding the route for presentation of antigen from intracellular sources such as from intracellular bacteria, which still remains unclear (Kulicke et al., 2020; McWilliam and Villadangos, 2018). However, the pathway for loading antigens from the extracellular milieu is much more well defined from several recent studies (Karamooz et al., 2019; McWilliam et al., 2016; McWilliam et al., 2020; Salio et al., 2020). Since the administration of novel MR1-targeting compounds will access MR1 from this route, here we will focus on this aspect of MR1 presentation (Figure 1).

In primary human cells, MR1 was found to reside almost entirely within the endoplasmic reticulum (ER). In this subcellular compartment, MR1 was found to be in an incompletely folded state, and the binding of antigen to a lysine residue (K43), via formation of a Schiff base, triggered a conformational change that allows the release and presentation of MR1-ligand complexes to the cell surface (McWilliam et al., 2016) (Figure 1). These data implied that the ligands could travel from outside the cell to the ER, rather than being captured within endosomes, which is the classical route of exogenous antigen loading on MHC class II or CD1 molecules. Recently, this route of antigen presentation was directly confirmed (McWilliam et al., 2020): a stable MR1-binding compound was synthesised with a fluorescent moiety attached (tetramethylrhodamine, TAMRA), that also functioned as an epitope tag (MR1 Antigen Analogue (MAgA)-TAMRA). This functionalised ligand revealed that in two cell lines, most MR1-ligand complexes formed in the ER, and in HeLa cells this occurred within

10 minutes of ligand exposure (McWilliam et al., 2020). This study also found that the efficiency of cells to capture and present MR1-antigen complexes was related to the number of ER-resident MR1 molecules, and that this pool of ligand-receptive molecules were maintained by the chaperone tapasin, and potentially tapasin-related protein (TAPBPR) (McWilliam et al., 2020). This confirmed that MR1 captures extracellular metabolites within the ER, an unexpected mode of detecting extracellular pathogens.

These results also suggest that the most efficient approach for modulating the MR1-MAIT cell axis would be to target novel compounds to the ER-resident, ligand receptive MR1 pool. How current ligands access the ER is unknown, but the canonical ligand 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) and MAgA-TAMRA do not cross the plasma membrane passively and were only imported into cells at 37 °C (McWilliam et al., 2020), suggesting an active transport mechanism of these metabolites. Such a transport mechanism may be a bottleneck for modified ligands and could be cell type specific – preventing the access to key antigen-presenting cells (APC). Hence synthetic ligands that circumvent transport by penetrating cell membranes passively, could be more universally active.

A second pathway for loading of MR1 has been shown to occur outside the ER, at the cell surface or in recycling endosomes. In this scenario, MR1 is recruited to the cell surface from the ER by a stabilizing ligand, then it is directed to an undefined location where ligand exchange occurs (Karamooz et al., 2019; McWilliam et al., 2016) (Figure 1). As formation of the Schiff base is required for efficient egress of MR1 from the ER, and its removal requires an acidic milieu, it is likely that the compartment reached after the cell surface is within the endo-lysosomal network. By this mechanism, Karamooz et al (2019) showed that the non-activating ligand 6-formyl pterin (6-FP) could enhance the ability of epithelial cells to activate MAIT cells by mycobacterial (*M. smegmatus*) supernatant, cell wall (H37Rv) or soluble 5-OP-

RU (Karamooz et al., 2019). The interpretation of these data is that 6-FP allows MR1 to egress to the surface and reach the endo-lysosomal network, where TCR activating antigens from mycobacteria bind to MR1.

This finding is surprising since 6-FP is usually associated with MAIT cells antagonism because it binds to MR1 efficiently but does not activate MAIT TCRs so can inhibit 5-OP-RU presentation by MR1 (Eckle et al., 2014). The discrepancy could be accounted for by the fact that the authors performed an overnight incubation with 6-FP, which was then removed. This experimental protocol led to the interpretation that by rescuing MR1 from degradation in the ER and promoting its accumulation at the cell surface (Karamooz et al., 2019), 6-FP also facilitated ligand exchange. Of note, this phenotype was more pronounced in epithelial cells that over-express MR1-GFP tagged molecules, which also had a prominent vacuolar MR1 localisation, suggestive of recycling. Consistently, knock-down of Syntaxin 4, affecting endosomal recycling, reduced the enhancing effect of 6-FP. Hence, exposure of cells to ligands that can potentially release MR1 from the ER is a mechanism for enhancing MR1 presentation. Although in wild type cells this mechanism does not seem dominant at steady state, it could play a role during infections and exposure to complex mixes of agonist and antagonist ligands, released with different kinetics or in different subcellular compartments.

Evidence for an endo-lysosomal pathway of MR1 antigen presentation was provided by a pro-drug agonist designed to overcome the instability of 5-OP-RU (Lange et al., 2020). In this pro-drug, the 5-amino group of 5-A-RU was modified with a cathepsin B cleavable valine-citrulline-p-aminobenzyl carbamate linker. The compound was efficiently presented to MAIT cells by myeloid APCs, and required lysosomal activity and MR1 recycling. Indeed, MAIT cell activation was abrogated by increasing concentrations of bafilomycin A1, an inhibitor of endosomal acidification by blocking vacuole ATPases, or by expression of GPI-linked MR1 molecules, which do not traffic deeply in the endo-lysosomal compartment (Salio et al., 2020).

In addition, and in agreement with results obtained pulsing epithelial cells with live mycobacteria (Karamooz et al., 2019), MAIT activation was not abrogated by simultaneous pulsing with Ac-6-FP, suggesting that Ac-6-FP and activating antigens are loading on a different pool of MR1 molecules (Lange et al., 2020). Interestingly, a short 10-minute exposure to the functionalised MAgA-TAMRA ligand also showed a punctate endosomal-like distribution (McWilliam et al., 2020), indicating that pinocytic uptake of MR1 ligands could render them suitable for lysosomal loading, depending on the biochemical properties of the compounds. However, since at steady state the majority of MR1 molecules reside in the ER (McWilliam et al., 2016), we believe that the ER route is likely to be the dominant route of soluble antigen presentation, which was directly observed (McWilliam et al., 2020), and any endosomal routes apparently requires some ligand to release MR1 from the ER.

Finally, a series of non-microbial MAIT cell agonists was recently identified through an *in-silico* screening of potential MR1 ligands (Salio et al., 2020). Several of these ‘DB series’ of agonists were shown to require internalization and endo-lysosomal loading for presentation, as demonstrated using THP1 expressing MR1-GPI linked molecules (Salio et al., 2020). However, in view of their weaker agonist activity, the physiological relevance of this series of compounds and of drug-like molecules previously reported to activate MAIT cells (Keller et al., 2017) remains to be determined.

In summary, our current understanding of the MR1 presentation pathway suggests that characteristics of effective ligands to modulate the MR1-MAIT cell axis include: ligands that are targeted to or access the ER and form a Schiff base with MR1; ligands that are able to passively cross membranes; ligands that reach the endo-lysosomal compartment and encounter MR1 molecules whose trafficking is controlled by other MR1 ligands; or ligands that liberate MR1 from the ER and can be exchanged by other, more scarce, ligands in distal compartments.



### 3. Recent advances in the design of synthetic MR1 antigens

The potent MR1 ligand, 5-OP-RU, can be readily synthesised, but in physiological conditions it is highly unstable with a half-life of only 88 min (Mak et al., 2017). 5-OP-RU has a single ring and a ketone group that forms the crucial covalent bond with MR1 (Figure 2). However, degradation of 5-OP-RU involves the intramolecular closure of this ketone group to form the bicyclic lumazine, 7-methyl-8-D-ribityllumazine (RL-7-Me), which cannot bond covalently with MR1 and behaves as weak agonist (Mak et al., 2017). To create a MAIT cell agonist without this inherent instability, Mak et al. (2017) synthesised a novel 5-OP-RU analogue by replacing just two nitrogen atoms with carbon. This prevented the cyclisation of the ketone group, forming a highly stable molecule, termed JYM72. No degradation was seen after 5 days under physiological conditions. In addition, JYM72 forms a Schiff base with K43, potentially upregulating MR1 at the cell surface with similar kinetics to the stable MAIT cell antagonist acetyl-6-formylpterin (Ac-6-FP) (Mak et al., 2017). Crucially, JYM72 is a bona fide MAIT cell agonist. In complex with MR1 it interacts with the MAIT TCR in a highly similar mode compared to 5-OP-RU (Awad et al., 2020) and induces human MAIT cell activation and cytokine production. When administered with the adjuvant CpG in mouse lungs, it caused the expansion and accumulation of MAIT cells (Mak et al., 2017). However, JYM72 is much less potent than 5-OP-RU, which is hypothesised to be due to an increased flexibility of its ribityl chain within the MR1 binding cleft (Awad et al., 2020). Other unknown factors may also influence its lack of potency, such as how efficiently it accesses MR1 within the ER. Nevertheless, JYM72 remains a highly promising stable MAIT cell agonist that has the ability to modulate MAIT cells *in vivo*.

Recently Awad and colleagues (2020) undertook a comprehensive examination of the molecular features that define potent MR1 ligands and MAIT cell agonists. They synthesised

a suite of novel compounds by modifying the ribityl chains of 5-OP-RU and RL-7-Me, which they termed ‘altered metabolite ligands’ (Awad et al., 2020; Ler et al., 2019). This revealed that the two correlates of potent MR1 surface upregulating compounds are the stability of the compound, and the ability to form a Schiff base with MR1 (Figure 2). Interestingly, the ribityl chain was not required, since a ‘ribityl-less analogue’ based on the JYM72 compound was the most powerful MR1-translocating ligand. Similarly, Ac-6-FP lacks the ribityl tail and induces potent MR1 upregulation (Eckle et al., 2015; McWilliam et al., 2016).

Yet for MAIT TCR stimulation, features on the ribityl chain were more important, as compounds lacking the 2’ and 3’ hydroxy groups reduced MAIT cell activation significantly, while removing those at positions 4’ and 5’ had little impact (Figure 2). Structurally, this was explained by potent agonists participating in an ‘interaction triad’ of hydrogen bonds, where the 2’ hydroxy group of the ribityl chain interacts with a tyrosine residue from the MAIT TCR (Y95 $\alpha$ ) which bonds with a tyrosine from MR1 (Y152) (Awad et al., 2020) (Figure 2). In two separate studies, the molecular features of the ribityl tail were also investigated by modifying the 5-OP-RU precursor, 5-amino-6-D-ribitylaminouracil (5-A-RU), or a ribityl lumazine (Braganza et al., 2020; Braganza et al., 2019). They showed similarly that the 2’-hydroxy was important for TCR stimulation.

Extending the finding that the ribityl chain is not required for a potent MR1 up-regulating ligand, and that the ribityl tail of 5-OP-RU reaches out of the MR1 antigen cleft, the first fluorescent MR1-binding ligand was synthesised, MAgA-TAMRA, as described above (McWilliam et al., 2020). Based on the JYM72 MR1-binding scaffold, the fluorescent TAMRA functional group replaced the ribityl chain on MAgA-TAMRA. This retained the stability and MR1 binding ability of JYM72, despite the additional bulky fluorophore. The availability of antibodies against TAMRA further added to the functionalisation of the TAMRA motif, by enabling the precipitation and localisation of MR1-MAgA-TAMRA complexes, and even the

detection of its presentation at the surface of cells expressing low endogenous levels of MR1 (McWilliam et al., 2020). Hence MAgA-TAMRA is a highly versatile tool to understand and directly monitor MR1 antigen presentation. It highlights the tolerance of MR1 ligand binding, and serves as a blueprint for creating novel functional MR1 ligands.

#### 4. MAIT cell antagonists

As mentioned above, the ribityl moiety of MR1 ligands has been linked to MAIT cell activation, while the formation of a Schiff base with K43 stabilises MR1-antigen complexes. A folic acid degradation product, 6-FP, was the first ligand shown to bind MR1 molecules (Kjer-Nielsen et al., 2012), yet it lacks agonist activity because it does not have the ribityl moiety. While 6-FP is a relatively weak inhibitor (Eckle et al., 2014), derivatives like Ac-6-FP and isobutyryl 6-FP, have been shown to compete with agonist ligands and dampen MR1 activation *in vitro* and *in vivo* (Corbett et al., 2014; Kjer-Nielsen et al., 2012; Murayama et al., 2019). These compounds potently upregulate MR1 surface expression, within hours of exposure, likely after gaining access to the cells and the ER in a mechanism still to be determined.

An unusual inhibitory mechanism has recently been described for compound DB28, and its derivative NV18.1, identified from an *in-silico* screening of putative MR1 ligands (Salio et al., 2020). DB28 is the first compound shown to reduce MR1 cell surface expression and to prevent newly synthesized molecules from reaching the cell surface. Accordingly, DB28-bound MR1 molecules are retained in the ER in an Endo-H sensitive conformation. Crystal studies revealed that DB28 binds the MR1 groove with an overall topology similarly to 5-OP-RU, and it is stabilised by hydrophobic interaction and hydrogen bonds with the arginine residues and K43 within the aromatic cradle. While DB28 is unable to form the Schiff base with K43, a K43A

mutation abrogates the ability of DB28 to retain MR1 in the ER, possibly because of rapid egress of the molecules towards the cell surface (McWilliam et al., 2016). Thus, DB28 and derivatives thereof, represent useful molecular tools to further our understanding of the molecular switch centred on K43 that triggers MR1 release from the ER (McWilliam et al., 2016). Intriguingly, weak agonist compounds like lumazines (Kjer-Nielsen et al., 2012; Patel et al., 2013) or diclofenac (Keller et al., 2017) also do not form a Schiff base, suggesting that loading can occur at the cell surface or in a recycling compartment, and possibly a different intracellular distribution of these ligands in comparison with DB28. The preferential inhibitory activity of DB28 on newly synthesized rather than ‘stored’ MR1 molecules (Salio et al., 2020) suggests the existence of pools of MR1 molecules in the ER with different accessibility, perhaps as a result of their association with components of the peptide loading complex (McWilliam et al., 2020).

## **5. Use of synthetic ligands *in vivo* to modulate MAIT cell activity**

Accumulating evidence suggests that in addition to their anti-microbial properties, MAIT cell can have wanted and unwanted effects in autoimmune, inflammatory and metabolic diseases (reviewed in Toubal et al. (2019)). Therefore, manipulation of the MR1 antigen presenting pathway with inhibitory ligands represents an attractive therapeutic target. As a proof of principle, in the autoimmune mouse model of systemic lupus erythematosus, where MAIT cells have a pathogenic role, the antagonist isobutyryl 6-FP has been shown to reduce autoantibody production and the severity of nephritis (Murayama et al., 2019). Similarly, in obese mice, where MAIT have been shown to contribute to adipose and intestinal inflammation, dysbiosis and metabolic alterations, treatment with Ac-6-FP reduces the chronic inflammation, reverses dysbiosis and improves metabolic control (Toubal et al., 2020). Lastly, the drug-related

molecule 3-F-SA has been shown to potently antagonise 5-OP-RU dependent MAIT cell expansion *in vivo* (Keller et al., 2017).

Conversely, where an increased activity of MAIT cells may be beneficial, their numbers can be boosted via agonists, often in conjunction with costimulatory signals. For example, intranasal injection of 5-OP-RU and toll-like receptor agonists (Pam2Cys, CpG or poly I:C) increases lung MAIT cell numbers ten-fold (Chen et al., 2017), and results in increased protection from *Legionella pneumoniae* infection (Wang et al., 2019). *In vivo* proliferation and activation of murine MAIT cells in the thymus and spleen can also be achieved via oral administration of 5-OP-RU (Legoux et al., 2019). We envisage that artificial expansion of MAIT cell numbers could be beneficial to control gastrointestinal infections, in the context of altered microbial flora, such as in HIV (Juno et al., 2019) or after allogeneic bone marrow transplantation (Dekker et al., 2020). In both situations, MAIT cells are reduced in frequencies, either as a consequence of altered gut permeability, or because of the conditioning regimen and the delayed reconstitution post-transplant.

## **6. Evasion of MR1-dependent antigen presentation**

Pathogens have evolved several strategies to subvert immune responses, including interfering with presentation of peptide antigens on MHC class I and class II molecules (reviewed in Tortorella et al. (2000)) and of lipids on CD1 molecules (Van Kaer and Joyce, 2006). Recently, it was shown that several herpesviruses (HSV, HCMV and MCMV) reduce basal MR1 surface expression in fibroblasts and prevent ligand induced MR1 upregulation, which might be a collateral effect of evolved responses to subvert MHC I or a parallel, targeted mechanism of MR1 downregulation (McSharry et al., 2020). One of the factors responsible for downregulation of MR1 expression in HSV infected cells was identified as the protein Us3,

however it remains to be determined which viral factors target MR1 for proteasomal degradation.

Although viruses do not have the vitamin B2 biosynthetic pathway and virus infected cells do not engage MAIT cells directly via their TCR, MAIT cells are activated via cytokines in several viral infections so are proposed to play an immunomodulatory role in signal amplification (reviewed in Ussher et al. (2018)). Given the ability of activated MAIT cells to mature dendritic cells through CD40L-dependent interactions (Salio et al., 2017), MR1 downregulation during viral infection could have important consequences for regulation of adaptive immunity and priming of antigen-specific T cells (Provine et al., 2019). Accordingly, a homozygous mutation abrogating MR1 antigen presentation was recently described in a young adult with recurrent viral and bacterial infections (Howson et al., 2020).

In addition, the microenvironment – microbial diversity, density, competition for oxygen and carbon sources – is known to influence bacterial metabolism and antigen availability (Schmaler et al., 2018; Tastan et al., 2018). Furthermore, antigen availability may differ across different isolates of the same pathogen, as shown for *Streptococcus pneumoniae*, resulting in differential MAIT cell activation (Hartmann et al., 2018; Kurioka et al., 2017). A novel mechanism of evasion from MAIT cell recognition has been reported for the African *Salmonella* typhimurium ST313 isolate that causes invasive disease (Preciado-Llanes et al., 2020). In this isolate, overexpression of *ribB*, encoding for a key enzyme in the riboflavin biosynthetic pathway, increases the overall amount of non-agonist metabolites such as riboflavin, flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), so that infected cells fail to effectively activate MAIT cells (Preciado-Llanes et al., 2020). Importantly, these results also suggest that weak MAIT cell agonists of the lumazine family (Corbett et al., 2014) do not accumulate at concentrations sufficient to elicit MAIT cell activation and are rapidly converted into non antigenic riboflavin, FMN, and FAD (Preciado-Llanes et al., 2020).

Lastly, like MHC class I and CD1d, MR1 is a  $\beta_2$ -microglobulin associated molecule and its surface expression is hampered by  $\beta_2$ -microglobulin loss, such as in DLD-1 cells (Miley et al., 2003). This could be an important bottleneck to harnessing MAIT and MR1-T cell function in cancers where loss of  $\beta_2$ -microglobulin is frequently reported, such as melanoma and colorectal cancers (Bernal et al., 2012).

## 7. Concluding remarks

Since the identification of the prototype MAIT cell ligands in 2012 (Kjer-Nielsen et al., 2012), great progress has been made in our understanding of the key steps that distinguish the MR1 from the classical MHC class I and II antigen presentation pathways. Still, we need to understand how soluble antigen enters the cells and is delivered to the ER for loading on nascent MR1 molecules. We need to consider and test how to facilitate antigen loading from in the endo-lysosomal compartment. The identification of MR1-restricted T cells recognising a number of tumours of different tissue origin, but not their normal cellular counterparts (Crowther et al., 2020; Lepore et al., 2017) now paves the way to attractive HLA-independent cancer immunotherapies against multiple cancers. However, the target antigen still requires careful identification to avoid unwanted toxicities as previously reported with engineered TCRs (Cameron et al., 2013).

## Figure legends

### Figure 1: Intracellular trafficking of MR1 informs novel ligand targeting approaches.

Antigen presenting cells (APC) expressing MR1 can be targeted by novel ligands to modulate antigen presentation. Schiff-base forming ligands targeting the endoplasmic reticulum (ER; 1) will release the ligand-receptive pool MR1 molecules towards the cell surface (A) for activation or inhibition of MR1-restricted T cells. Some ligands can block the exit of MR1

from the ER (B) such as the compound DB28. An alternative route is for ligands to be targeted to the endosomal compartment (2) for recycling back to the cell surface (C).

## **Figure 2: Key features of the potent agonist MR1 ligand, 5-OP-RU**

The riboflavin-derived MR1 ligand 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) possesses a crucial ketone group that interacts with MR1 lysine 43 (K43), and a ribityl tail (green). This has four hydroxy groups that have varying degrees of importance for T cell receptor (TCR) stimulation; the most important is that at position 2', which interacts with the TCR tyrosine 95 $\alpha$  (Y95 $\alpha$ ) which in turn bonds with MR1 tyrosine 152 (Y152).

## **Acknowledgements**

HMCW is supported by a Faculty Research Fellowship from the Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne. MS acknowledges support from the Medical Research Council (MRC) Human Immunology Unit Core Funding.

## **Conflict of interests**

MS receives consulting fees from Nucleome Therapeutics.

## **Author contributions**

Both authors wrote the manuscript and approved publication.



## 388    **References**

- 389    Awad, W., Ler, G.J.M., Xu, W., Keller, A.N., Mak, J.Y.W., Lim, X.Y., Liu, L., Eckle,  
390    S.B.G., Le Nours, J., McCluskey, J., Corbett, A.J., Fairlie, D.P., Rossjohn, J., 2020. The  
391    molecular basis underpinning the potency and specificity of MAIT cell antigens. *Nature*  
392    *Immunology* 21, 400-411.
- 393    Bernal, M., Ruiz-Cabello, F., Concha, A., Paschen, A., Garrido, F., 2012. Implication of the  
394     $\beta$ 2-microglobulin gene in the generation of tumor escape phenotypes. *Cancer Immunology,*  
395    *Immunotherapy* 61, 1359-1371.
- 396    Braganza, C.D., Motozono, C., Sonoda, K.H., Yamasaki, S., Shibata, K., Timmer, M.S.M.,  
397    Stocker, B.L., 2020. Agonistic or antagonistic mucosal-associated invariant T (MAIT) cell  
398    activity is determined by the 6-alkylamino substituent on uracil MR1 ligands. *Chemical*  
399    *Communications* 56, 5291-5294.
- 400    Braganza, C.D., Shibata, K., Fujiwara, A., Motozono, C., Sonoda, K.H., Yamasaki, S.,  
401    Stocker, B.L., Timmer, M.S.M., 2019. The effect of MR1 ligand glyco-analogues on  
402    mucosal-associated invariant T (MAIT) cell activation. *Organic & Biomolecular Chemistry*  
403    17, 8992-9000.
- 404    Cameron, B.J., Gerry, A.B., Dukes, J., Harper, J.V., Kannan, V., Bianchi, F.C., Grand, F.,  
405    Brewer, J.E., Gupta, M., Plesa, G., Bossi, G., Vuidepot, A., Powlesland, A.S., Legg, A.,  
406    Adams, K.J., Bennett, A.D., Pumphrey, N.J., Williams, D.D., Binder-Scholl, G.,  
407    Kulikovskaya, I., Levine, B.L., Riley, J.L., Varela-Rohena, A., Stadtmayer, E.A., Rapoport,  
408    A.P., Linette, G.P., June, C.H., Hassan, N.J., Kalos, M., Jakobsen, B.K., 2013. Identification  
409    of a Titin-Derived HLA-A1–Presented Peptide as a Cross-Reactive Target for Engineered  
410    MAGE A3–Directed T Cells. *Science Translational Medicine* 5, 197ra103-197ra103.
- 411    Chen, Z., Wang, H., D'Souza, C., Sun, S., Kostenko, L., Eckle, S.B., Meehan, B.S., Jackson,  
412    D.C., Strugnell, R.A., Cao, H., Wang, N., Fairlie, D.P., Liu, L., Godfrey, D.I., Rossjohn, J.,  
413    McCluskey, J., Corbett, A.J., 2017. Mucosal-associated invariant T-cell activation and  
414    accumulation after in vivo infection depends on microbial riboflavin synthesis and co-  
415    stimulatory signals. *Mucosal immunology* 10, 58-68.
- 416    Constantinides, M.G., Link, V.M., Tamoutounour, S., Wong, A.C., Perez-Chaparro, P.J.,  
417    Han, S.-J., Chen, Y.E., Li, K., Farhat, S., Weckel, A., Krishnamurthy, S.R., Vujkovic-Cvijin,  
418    I., Linehan, J.L., Bouladoux, N., Merrill, E.D., Roy, S., Cua, D.J., Adams, E.J., Bhandoola,  
419    A., Scharschmidt, T.C., Aubé, J., Fischbach, M.A., Belkaid, Y., 2019. MAIT cells are  
420    imprinted by the microbiota in early life and promote tissue repair. *Science* 366, eaax6624.
- 421    Corbett, A.J., Eckle, S.B., Birkinshaw, R.W., Liu, L., Patel, O., Mahony, J., Chen, Z.,  
422    Reantragoon, R., Meehan, B., Cao, H., Williamson, N.A., Strugnell, R.A., Van Sinderen, D.,  
423    Mak, J.Y., Fairlie, D.P., Kjer-Nielsen, L., Rossjohn, J., McCluskey, J., 2014. T-cell activation  
424    by transitory neo-antigens derived from distinct microbial pathways. *Nature* 509, 361-365.
- 425    Crowther, M.D., Dolton, G., Legut, M., Caillaud, M.E., Lloyd, A., Attaf, M., Galloway,  
426    S.A.E., Rius, C., Farrell, C.P., Szomolay, B., Ager, A., Parker, A.L., Fuller, A., Donia, M.,  
427    McCluskey, J., Rossjohn, J., Svane, I.M., Phillips, J.D., Sewell, A.K., 2020. Genome-wide  
428    CRISPR-Cas9 screening reveals ubiquitous T cell cancer targeting via the monomorphic  
429    MHC class I-related protein MR1. *Nat Immunol* 21, 178-185.

430 Dekker, L., De Koning, C., Lindemans, C., Nierkens, S., 2020. Reconstitution of T Cell  
431 Subsets Following Allogeneic Hematopoietic Cell Transplantation. *Cancers* 12, 1974.

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

435 Eckle, S.B., Birkinshaw, R.W., Kostenko, L., Corbett, A.J., McWilliam, H.E., Reantragoon,  
436 R., Chen, Z., Gherardin, N.A., Beddoe, T., Liu, L., Patel, O., Meehan, B., Fairlie, D.P.,  
437 Villadangos, J.A., Godfrey, D.I., Kjer-Nielsen, L., McCluskey, J., Rossjohn, J., 2014. A  
438 molecular basis underpinning the T cell receptor heterogeneity of mucosal-associated  
439 invariant T cells. *J Exp Med* 211, 1585-1600.

440 Eckle, S.B., Corbett, A.J., Keller, A., Chen, Z., Godfrey, D.I., Liu, L., Mak, J.Y., Fairlie,  
441 D.P., Rossjohn, J., McCluskey, J., 2015. Recognition of Vitamin B precursors and  
442 byproducts by Mucosal Associated Invariant T cells. *J Biol Chem* 290, 30204-30211.

443 Gold, M.C., Cerri, S., Smyk-Pearson, S., Cansler, M.E., Vogt, T.M., Delepine, J., Winata, E.,  
444 Swarbrick, G.M., Chua, W.J., Yu, Y.Y., Lantz, O., Cook, M.S., Null, M.D., Jacoby, D.B.,  
445 Harriff, M.J., Lewinsohn, D.A., Hansen, T.H., Lewinsohn, D.M., 2010. Human mucosal  
446 associated invariant T cells detect bacterially infected cells. *PLoS biology* 8, e1000407.

447 Hartmann, N., McMurtrey, C., Sorensen, M.L., Huber, M.E., Kurapova, R., Coleman, F.T.,  
448 Mizgerd, J.P., Hildebrand, W., Kronenberg, M., Lewinsohn, D.M., Harriff, M.J., 2018.  
449 Riboflavin Metabolism Variation among Clinical Isolates of *Streptococcus pneumoniae*  
450 Results in Differential Activation of Mucosal-associated Invariant T Cells. *American Journal*  
451 *of Respiratory Cell and Molecular Biology* 58, 767-776.

452 Hashimoto, K., Hirai, M., Kurosawa, Y., 1995. A gene outside the human MHC related to  
453 classical HLA class I genes. *Science* 269, 693-695.

454 Hinks, T.S.C., Marchi, E., Jabeen, M., Olshansky, M., Kurioka, A., Pediongco, T.J., Meehan,  
455 B.S., Kostenko, L., Turner, S.J., Corbett, A.J., Chen, Z., Klenerman, P., McCluskey, J., 2019.  
456 Activation and *In Vivo* Evolution of the MAIT Cell Transcriptome in Mice and Humans  
457 Reveals Tissue Repair Functionality. *Cell reports* 28, 3249-3262.e3245.

458 Howson, L.J., Awad, W., von Borstel, A., Lim, H.J., McWilliam, H.E.G., Sandoval-Romero,  
459 M.L., Majumdar, S., Hamzeh, A.R., Andrews, T.D., McDermott, D.H., Murphy, P.M., Le  
460 Nours, J., Mak, J.Y.W., Liu, L., Fairlie, D.P., McCluskey, J., Villadangos, J.A., Cook, M.C.,  
461 Turner, S.J., Davey, M.S., Ojaimi, S., Rossjohn, J., 2020. Absence of mucosal-associated  
462 invariant T cells in a person with a homozygous point mutation in MR1. *Sci Immunol* 5.

463 Juno, J.A., Phetsouphanh, C., Klenerman, P., Kent, S.J., 2019. Perturbation of mucosal-  
464 associated invariant T cells and iNKT cells in HIV infection. *Current Opinion in HIV and*  
465 *AIDS* 14, 77-84.

466 Karamooz, E., Harriff, M.J., Narayanan, G.A., Worley, A., Lewinsohn, D.M., 2019. MR1  
467 recycling and blockade of endosomal trafficking reveal distinguishable antigen presentation  
468 pathways between *Mycobacterium tuberculosis* infection and exogenously delivered  
469 antigens. *Scientific Reports* 9.

470 Keller, A.N., Eckle, S.B., Xu, W., Liu, L., Hughes, V.A., Mak, J.Y., Meehan, B.S.,  
471 Pediongco, T., Birkinshaw, R.W., Chen, Z., Wang, H., D'Souza, C., Kjer-Nielsen, L.,  
472 Gherardin, N.A., Godfrey, D.I., Kostenko, L., Corbett, A.J., Purcell, A.W., Fairlie, D.P.,  
473 McCluskey, J., Rossjohn, J., 2017. Drugs and drug-like molecules can modulate the function  
474 of mucosal-associated invariant T cells. *Nat Immunol* 18, 402-411.

475 Kjer-Nielsen, L., Patel, O., Corbett, A.J., Le Nours, J., Meehan, B., Liu, L., Bhati, M., Chen,  
476 Z., Kostenko, L., Reantragoon, R., Williamson, N.A., Purcell, A.W., Dudek, N.L.,  
477 McConville, M.J., O'Hair, R.A., Khairallah, G.N., Godfrey, D.I., Fairlie, D.P., Rossjohn, J.,  
478 McCluskey, J., 2012. MR1 presents microbial vitamin B metabolites to MAIT cells. *Nature*  
479 491, 717-723.

480 Kulicke, C., Karamooz, E., Lewinsohn, D., Harrieff, M., 2020. Covering All the Bases:  
481 Complementary MR1 Antigen Presentation Pathways Sample Diverse Antigens and  
482 Intracellular Compartments. *Frontiers in Immunology* 11.

483 Kurioka, A., van Wilgenburg, B., Javan, R.R., Hoyle, R., van Tonder, A.J., Harrold, C.L.,  
484 Leng, T., Howson, L.J., Shepherd, D., Cerundolo, V., Brueggemann, A.B., Klenerman, P.,  
485 2017. Diverse *Streptococcus pneumoniae* strains drive a MAIT cell response through MR1-  
486 dependent and cytokine-driven pathways. *J Infect Dis*.

487 Lamichhane, R., Schneider, M., de la Harpe, S.M., Harrop, T.W.R., Hannaway, R.F.,  
488 Dearden, P.K., Kirman, J.R., Tyndall, J.D.A., Vernall, A.J., Ussher, J.E., 2019. TCR- or  
489 Cytokine-Activated CD8<sup>+</sup> Mucosal-Associated Invariant T Cells Are Rapid  
490 Polyfunctional Effectors That Can Coordinate Immune Responses. *Cell reports* 28, 3061-  
491 3076.e3065.

492 Lange, J., Anderson, R.J., Marshall, A.J., Chan, S.T.S., Bilbrough, T.S., Gasser, O.,  
493 Gonzalez-Lopez, C., Salio, M., Cerundolo, V., Hermans, I.F., Painter, G.F., 2020. The  
494 Chemical Synthesis, Stability, and Activity of MAIT Cell Prodrug Agonists That Access  
495 MR1 in Recycling Endosomes. *ACS Chem Biol* 15, 437-445.

496 Le Bourhis, L., Dusseaux, M., Bohineust, A., Bessoles, S., Martin, E., Premel, V., Core, M.,  
497 Sleurs, D., Serriari, N.E., Treiner, E., Hivroz, C., Sansonetti, P., Gougeon, M.L., Soudais, C.,  
498 Lantz, O., 2013. MAIT Cells Detect and Efficiently Lyse Bacterially-Infected Epithelial  
499 Cells. *PLoS pathogens* 9, e1003681.

500 Le Bourhis, L., Martin, E., Peguillet, I., Guihot, A., Froux, N., Core, M., Levy, E., Dusseaux,  
501 M., Meyssonier, V., Premel, V., Ngo, C., Riteau, B., Duban, L., Robert, D., Huang, S.,  
502 Rottman, M., Soudais, C., Lantz, O., 2010. Antimicrobial activity of mucosal-associated  
503 invariant T cells. *Nat Immunol* 11, 701-708.

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

508 Leng, T., Akther, H.D., Hackstein, C.P., Powell, K., King, T., Friedrich, M., Christoforidou,  
509 Z., McCuaig, S., Neyazi, M., Arancibia-Carcamo, C.V., Hagel, J., Powrie, F., Oxford,  
510 I.B.D.I., Peres, R.S., Millar, V., Ebner, D., Lamichhane, R., Ussher, J., Hinks, T.S.C.,  
511 Marchi, E., Willberg, C., Klenerman, P., 2019. TCR and Inflammatory Signals Tune Human

512 MAIT Cells to Exert Specific Tissue Repair and Effector Functions. *Cell reports* 28, 3077-  
513 3091 e3075.

514 Lepore, M., Kalinichenko, A., Calogero, S., Kumar, P., Paleja, B., Schmalzer, M., Narang, V.,  
515 Zolezzi, F., Poidinger, M., Mori, L., De Libero, G., 2017. Functionally diverse human T cells  
516 recognize non-microbial antigens presented by MR1. *Elife* 6:e24476.

517 Ler, G.J.M., Xu, W., Mak, J.Y.W., Liu, L., Bernhardt, P.V., Fairlie, D.P., 2019. Computer  
518 modelling and synthesis of deoxy and monohydroxy analogues of a ribitylaminouracil  
519 bacterial metabolite that potently activates human T cells. *Chemistry* 25, 15594-15608.

520 Mak, J.Y., Xu, W., Reid, R.C., Corbett, A.J., Meehan, B.S., Wang, H., Chen, Z., Rossjohn, J.,  
521 McCluskey, J., Liu, L., Fairlie, D.P., 2017. Stabilizing short-lived Schiff base derivatives of  
522 5-aminouracils that activate mucosal-associated invariant T cells. *Nat Commun* 8, 14599.

523 McSharry, B.P., Samer, C., McWilliam, H.E.G., Ashley, C.L., Yee, M.B., Steain, M., Liu, L.,  
524 Fairlie, D.P., Kinchington, P.R., McCluskey, J., Abendroth, A., Villadangos, J.A., Rossjohn,  
525 J., Slobedman, B., 2020. Virus-Mediated Suppression of the Antigen Presentation Molecule  
526 MR1. *Cell reports* 30, 2948-2962 e2944.

527 McWilliam, H.E., Eckle, S.B., Theodossis, A., Liu, L., Chen, Z., Wubben, J.M., Fairlie, D.P.,  
528 Strugnell, R.A., Minter, J.D., McCluskey, J., Rossjohn, J., Villadangos, J.A., 2016. The  
529 intracellular pathway for the presentation of vitamin B-related antigens by the antigen-  
530 presenting molecule MR1. *Nat Immunol* 17, 531-537.

531 McWilliam, H.E., Mak, J.Y., Awad, W., Zorkau, M., Cruz-Gomez, S., Lim, H.J., Yan, Y.,  
532 Wormald, S., Dagley, L.F., Eckle, S.B., Corbett, A., Liu, H., Li, S., Reddiex, S.J.J., Minter,  
533 J.D., Liu, L., McCluskey, J., Rossjohn, J., Fairlie, D.P., Villadangos, J.A., 2020. Endoplasmic  
534 Reticulum Chaperones Stabilize Ligand-Receptive MR1 molecules for Efficient Presentation  
535 of Metabolite Antigens. *Proceedings of the National Academy of Sciences* 117, 24974-  
536 24985.

537 McWilliam, H.E., Villadangos, J.A., 2018. MR1 antigen presentation to MAIT cells: new  
538 ligands, diverse pathways? *Curr Opin Immunol* 52, 108-113.

539 Meierovics, Cowley, S.C., 2016. MAIT cells promote inflammatory monocyte differentiation  
540 into dendritic cells during pulmonary intracellular infection. *J Exp Med* 213, 2793-2809.

541 Meierovics, Yankelevich, W.J., Cowley, S.C., 2013. MAIT cells are critical for optimal  
542 mucosal immune responses during in vivo pulmonary bacterial infection. *Proc Natl Acad Sci*  
543 U S A 110, E3119-3128.

544 Miley, M.J., Truscott, S.M., Yu, Y.Y., Gilfillan, S., Fremont, D.H., Hansen, T.H., Lybarger,  
545 L., 2003. Biochemical features of the MHC-related protein 1 consistent with an  
546 immunological function. *J Immunol* 170, 6090-6098.

547 Murayama, G., Chiba, A., Suzuki, H., Nomura, A., Mizuno, T., Kuga, T., Nakamura, S.,  
548 Amano, H., Hirose, S., Yamaji, K., Suzuki, Y., Tamura, N., Miyake, S., 2019. A Critical  
549 Role for Mucosal-Associated Invariant T Cells as Regulators and Therapeutic Targets in  
550 Systemic Lupus Erythematosus. *Frontiers in Immunology* 10.

551 Patel, O., Kjer-Nielsen, L., Le Nours, J., Eckle, S.B.G., Birkinshaw, R., Beddoe, T., Corbett,  
 552 A.J., Liu, L., Miles, J.J., Meehan, B., Reantragoon, R., Sandoval-Romero, M.L., Sullivan,  
 553 L.C., Brooks, A.G., Chen, Z., Fairlie, D.P., McCluskey, J., Rossjohn, J., 2013. Recognition of  
 554 vitamin B metabolites by mucosal-associated invariant T cells. *Nature Communications* 4.

555 Preciado-Llanes, L., Aulicino, A., Canals, R., Moynihan, P.J., Zhu, X., Jambo, N., Nyirenda,  
 556 T.S., Kadwala, I., Sousa Gerós, A., Owen, S.V., Jambo, K.C., Kumwenda, B., Veerapen, N.,  
 557 Besra, G.S., Gordon, M.A., Hinton, J.C.D., Napolitani, G., Salio, M., Simmons, A., 2020.  
 558 Evasion of MAIT cell recognition by the African *Salmonella Typhimurium* ST313 pathovar  
 559 that causes invasive disease. *Proceedings of the National Academy of Sciences*, 202007472.

560 Provine, N.M., Amini, A., Garner, L.C., Dold, C., Hutchings, C., FitzPatrick, M.E.B., Reyes,  
 561 L.S., Chinnakannan, S., Oguti, B., Raymond, M., Capone, S., Folgori, A., Rollier, C.S.,  
 562 Barnes, E., Pollard, A.J., Klenerman, P., 2019. Activation of MAIT cells plays a critical role  
 563 in viral vector vaccine immunogenicity. *bioRxiv*, 661397.

564 Rock, K.L., Reits, E., Neefjes, J., 2016. Present Yourself! By MHC Class I and MHC Class II  
 565 Molecules. *Trends Immunol* 37, 724-737.

566 Salio, M., Awad, W., Veerapen, N., Gonzalez-Lopez, C., Kulicke, C., Waithe, D., Martens,  
 567 A.W.J., Lewinsohn, D.M., Hobrath, J.V., Cox, L.R., Rossjohn, J., Besra, G.S., Cerundolo, V.,  
 568 2020. Ligand-dependent downregulation of MR1 cell surface expression. *Proc Natl Acad Sci*  
 569 U S A 117, 10465-10475.

570 Salio, M., Gasser, O., Gonzalez-Lopez, C., Martens, A., Veerapen, N., Gileadi, U., Verter,  
 571 J.G., Napolitani, G., Anderson, R., Painter, G., Besra, G.S., Hermans, I.F., Cerundolo, V.,  
 572 2017. Activation of Human Mucosal-Associated Invariant T Cells Induces CD40L-  
 573 Dependent Maturation of Monocyte-Derived and Primary Dendritic Cells. *J Immunol* 199,  
 574 2631-2638.

575 Schmalzer, M., Colone, A., Spagnuolo, J., Zimmermann, M., Lepore, M., Kalinichenko, A.,  
 576 Bhatia, S., Cottier, F., Rutishauser, T., Pavelka, N., Egli, A., Azzali, E., Pieroni, M.,  
 577 Costantino, G., Hruz, P., Sauer, U., Mori, L., De Libero, G., 2018. Modulation of bacterial  
 578 metabolism by the microenvironment controls MAIT cell stimulation. *Mucosal immunology*.

579 Seshadri, C., Thuong, N.T.T., Mai, N.T.H., Bang, N.D., Chau, T.T.H., Lewinsohn, D.M.,  
 580 Thwaites, G.E., Dunstan, S.J., Hawn, T.R., 2016. A polymorphism in human MR1 is  
 581 associated with mRNA expression and susceptibility to tuberculosis. *Genes Immun*.

582 Soudais, C., Samassa, F., Sarkis, M., Le Bourhis, L., Bessoles, S., Blanot, D., Herve, M.,  
 583 Schmidt, F., Mengin-Lecreulx, D., Lantz, O., 2015. In Vitro and In Vivo Analysis of the  
 584 Gram-Negative Bacteria-Derived Riboflavin Precursor Derivatives Activating Mouse MAIT  
 585 Cells. *J Immunol* 194, 4641-4649.

586 Souter, M.N.T., Eckle, S.B.G., 2020. Biased MAIT TCR Usage Poised for Limited Antigen  
 587 Diversity? *Frontiers in Immunology* 11.

588 Tastan, C., Karhan, E., Zhou, W., Fleming, E., Voigt, A.Y., Yao, X., Wang, L., Horne, M.,  
 589 Placek, L., Kozhaya, L., Oh, J., Unutmaz, D., 2018. Tuning of human MAIT cell activation  
 590 by commensal bacteria species and MR1-dependent T-cell presentation. *Mucosal*  
 591 *immunology* 11, 1591-1605.

592 Tortorella, D., Gewurz, B.E., Furman, M.H., Schust, D.J., Ploegh, H.L., 2000. Viral  
593 subversion of the immune system. *Annu Rev Immunol* 18, 861-926.

594 Toubal, A., Kiaf, B., Beaudoin, L., Cagninacci, L., Rhimi, M., Fruchet, B., Da Silva, J.,  
595 Corbett, A.J., Simoni, Y., Lantz, O., Rossjohn, J., McCluskey, J., Lesnik, P., Maguin, E.,  
596 Lehuen, A., 2020. Mucosal-associated invariant T cells promote inflammation and intestinal  
597 dysbiosis leading to metabolic dysfunction during obesity. *Nature Communications* 11.

598 Toubal, A., Nel, I., Lotersztajn, S., Lehuen, A., 2019. Mucosal-associated invariant T cells  
599 and disease. *Nature Reviews Immunology* 19, 643-657.

600 Ussher, J.E., Willberg, C.B., Klenerman, P., 2018. MAIT cells and viruses. *Immunology and*  
601 *Cell Biology* 96, 630-641.

602 Van Kaer, L., Joyce, S., 2006. Viral evasion of antigen presentation: not just for peptides  
603 anymore. *Nature Immunology* 7, 795-797.

604 Walker, L.J., Kang, Y.H., Smith, M.O., Tharmalingham, H., Ramamurthy, N., Fleming,  
605 V.M., Sahgal, N., Leslie, A., Oo, Y., Geremia, A., Scriba, T.J., Hanekom, W.A., Lauer,  
606 G.M., Lantz, O., Adams, D.H., Powrie, F., Barnes, E., Klenerman, P., 2012. Human MAIT  
607 and CD8<sup>+</sup> cells develop from a pool of type-17 precommitted CD8<sup>+</sup> T cells. *119*, 422-433.

608 Wang, H., Kjer-Nielsen, L., Shi, M., D'Souza, C., Pediongco, T.J., Cao, H., Kostenko, L.,  
609 Lim, X.Y., Eckle, S.B.G., Meehan, B.S., Zhu, T., Wang, B., Zhao, Z., Mak, J.Y.W., Fairlie,  
610 D.P., Teng, M.W.L., Rossjohn, J., Yu, D., De St Groth, B.F., Lovrecz, G., Lu, L.,  
611 McCluskey, J., Strugnell, R.A., Corbett, A.J., Chen, Z., 2019. IL-23 costimulates antigen-  
612 specific MAIT cell activation and enables vaccination against bacterial infection. *Science*  
613 *Immunology* 4, eaaw0402.

614