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MR1 antigen presentation to MAIT cells: new ligands, diverse pathways?

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The major histocompatibility complex class I-related molecule MR1 is the only antigen (Ag) presenting molecule that captures and displays vitamin B-derived metabolites that are unique to a wide array of microbes. Presentation of these metabolite Ag at the cell surface activates mucosal-associated invariant T (MAIT) cells, a highly abundant innate-like T cell population, and represents a recently-described mechanism used by the mammalian immune system to sense pathogenic or commensal microbes. Our understanding of the cell biology of how MR1 accomplishes this unique task is still evolving, but recent advances are allowing a general picture to emerge. Further, the list of metabolites presented by MR1 is expanding both by identification of natural metabolites and the design of synthetic ligands. Here we review the latest studies contributing to our growing understanding of this rapidly expanding field.

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Introduction

Presentation of antigen (Ag) by MHC molecules to T cells is a critical step of adaptive immunity, culminating in formation of effector T lymphocytes required for pathogen clearance. Distinct T cell populations recognise different classes of Ag bound to specialized Ag-presenting molecules. By contrast to conventional T cells, which collectively recognise an almost limitless array of peptides presented by classical major histocompatibility complex (MHC) molecules, innate-like T cells recognise more conserved non-protein derived Ag presented by non-classical MHC class I-like molecules such as the CD1 family or MHC class I-related protein 1 (MR1). MR1 is unique in its ability to capture and present transient metabolites derived from the microbial synthesis of vitamin B2, riboflavin, to a highly abundant innate-like T cell population known as mucosal-associated invariant T (MAIT) cells [1,2]. Many bacteria and yeasts synthesise riboflavin, unlike mammals, hence a precursor 'building block' of riboflavin has been co-opted by the mammalian immune system as a metabolic signature for the presence of microbes. These vitamin B2-derived Ag (VitBAg) are therefore akin to a pathogen-associated molecular pattern representing a diverse range of microbes [3]. Once activated, MAIT cells can secrete inflammatory cytokines, directly kill cells presenting MR1–ligand complexes and accumulate in infected tissues [4], thus participating in protection against several bacterial infections [5]. Whereas the role of MR1 in MAIT stimulation has been known for 15 years [6], the antigen-presentation pathway is the least well understood aspect and many key questions remain open. This review will describe the current understanding of the cell biology of MR1 Ag-presentation and the growing list of its unique metabolite ligands presented which implies an expanding role for the MR1–MAIT cell axis.

MR1 ligands

The known MR1 ligands encompass a range of natural and synthetic metabolites of two broad classes; those that activate MAIT cells derived from vitamin B2, and those that do not that are vitamin B9-related (Table 1). The most potent natural MAIT-activating VitBAg described so far is the single-ring pyrimidine compound 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU), formed from the spontaneous reaction between the riboflavin precursor 5-amino-6-D-ribitylaminouracil (5-A-RU) with methylglyoxal, a small metabolite of glycolysis [1]. Other slightly less-active pyrimidine Ag are formed when 5-A-RU combines with glyoxal or di-hydroxy acetone [1,7•]. A second sub-class of MAIT-activating ligands, much less potent than the pyrimidines, are the double-ringed ribityl lumazine compounds [2]. An important distinction between the pyrimidines and ribityl lumazines is that while both types of ligands establish non-covalent interactions with the MR1 Ag binding site that contribute to stabilise the resulting complex, only the pyrimidine ligands form a covalent bond with MR1, specifically a Schiff base with MR1 residue 43, a lysine [1].

Table 1

The major classes of MR1 metabolite ligands currently known.

MR1 ligand	Vitamin family relation	MAIT cell activation	Cell-surface MR1 up-regulation activity	Schiff base formation with MR1	References
Pyrimidines (e.g. 5-OP-RU)	Vitamin B2	+++	+++	+	[1,15••]
Ribityl lumazines	Vitamin B2	+	–	–	[1,2]
Formyl pterins (e.g. 6-FP)	Vitamin B9	–	++	+	[2]

Formation of this covalent Schiff base bond is a critical step in the trafficking of MR1–VitBAg complexes, discussed later, and explains the large difference in the potency of the two pyrimidine Ag sub-classes [8]. Indeed, the contribution of the ribityl lumazines to anti-microbial MAIT cell activation is questionable [9]. Using bacteria deficient in each riboflavin synthesis pathway, it has been shown that while the ability to produce 5-A-RU is critical for MAIT activation, loss of ribityl lumazine synthesis did not affect activation by either *Lactobacillus lactis* or *Escherichia coli* [1,7•].

The second class of known MR1 ligands, those that do not activate MAIT cells, are derived from the degradation products of vitamin B9, folic acid, and include 6-formyl pterin (6-FP), and its acetylated form, acetyl-6-FP. Both bind to MR1 via formation of a Schiff base with Lys 43 and are efficiently presented at the cell surface, but they do not stimulate the MAIT TCR [2,10]. Rather, they can inhibit MAIT recognition of riboflavin-derived VitBAg by competing for MR1 binding.

In summary, to potently induce MAIT cell responses, MR1 ligands must fulfil two conditions. Firstly, to form stable complexes with MR1, which is facilitated by formation of a Schiff base bond with MR1 lysine 43. The second is to possess a structure that can be recognised by MAIT TCRs when exposed on the cell surface as a MR1–VitBAg complex. In the next section we describe what is known about the pathway for presentation of these ligands.

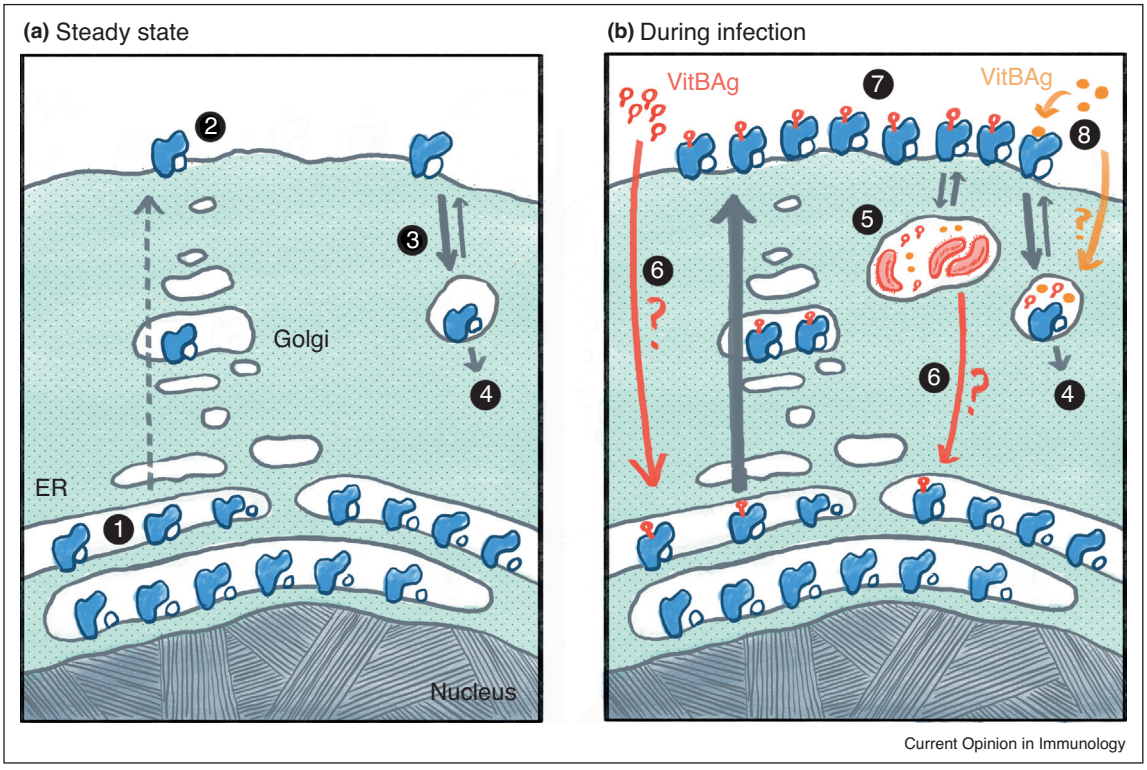
MR1 as an ER-resident sensor of microbial vitamin metabolites

The *Mr1* gene was discovered in 1995 [11], but the identity of the MR1 ligands was not published until 2012 [2]. Valuable but limited insights into MR1 biology were obtained in the intervening years (reviewed in [3]), but the ability to synthesise VitBAgs [12••] or to obtain them from commercial sources is causing a rapid expansion of this field. Critical aspects can now be addressed, such as the cellular location where MR1–VitBAg complexes form. While our understanding is still evolving and some discrepancies are evident, a general picture of the MR1 presentation pathway is emerging. While MR1 behaves according to general principles shared with classical MHC and CD1 molecules, there are also significant differences that make the MR1 presentation pathway as distinct as the ligands it presents.

A striking feature of MR1 is that although it is ubiquitously transcribed and synthesised, its level of expression at the cell surface in the absence of pathogen infections is very low [13]. This is counterintuitive for an Ag-presenting molecule, since transcription and surface expression of classical MHC or CD1 molecules largely correlate [14]. Now we know this is because the majority of MR1 is sequestered within the endoplasmic reticulum (ER) in the absence of ligand [15••,16–19]. A simple way to show this biochemically is by treating immunoprecipitated MR1 molecules with endoglycosidase H (Endo H), which cleaves sugars from glycoprotein that have not yet crossed the golgi in their way to the plasma membrane or endocytic route. We found that almost all MR1 molecules in primary human peripheral blood mononuclear cells had 'Endo H-sensitive' glycans, indicating that in the steady state these molecules have not yet progressed through the Golgi apparatus [15••]. These observations raise two questions. Firstly, do the ER-resident molecules bind any endogenous ligands in the steady state? This is unknown, but we do know that the MR1 molecules residing in the ER retain a 'ligand-receptive' conformation, different from the conformation of loaded molecules expressed on the cell surface [20]. This favours the hypothesis that the ER-resident molecules are in fact empty. The second question is, what protects these empty molecules from ER-associated degradation (ERAD)? Manipulations that prevent cytosolic peptide antigens from reaching the ER for MHC I loading cause rapid degradation of MHC I molecules, except for a small proportion that escapes to the cell surface with no or suboptimal ligands [21]. This is not the fate of empty MR1, pointing to the existence of a 'VitB loading complex' (VBLC) which, analogous to the MHC I peptide loading complex [14], stabilises empty MR1 and protects it from premature degradation via ERAD. The composition of this hypothetical VBLC remains unknown.

When and how is MR1 recruited to the cell surface for presentation to MAIT cells? Some studies have found that inflammatory signals can moderately increase surface MR1 (see below), but it has long been known that the most potent cue which causes intracellular MR1 to traffic from the ER to the cell surface is the presence of

Figure 1



A model of how MR1 presents microbial antigens. **(a)** In the absence of infection or antigen, immature MR1 molecules (blue) remain inside the endoplasmic reticulum (ER) weakly associated with β-2-microglobulin (white) (1). A small cohort of MR1 molecules can exit the ER stochastically to follow the secretory pathway to the cell surface (2), where they are eventually internalised (3) and degraded in the endosomal compartment (4). **(b)** During infection vitamin B-derived Ag (VitBAg) is available outside or inside the cell (5). VitBAg capable of forming a Schiff-base with MR1 (red) enters the cell compartments including the ER via an unknown process (6). This releases MR1 from the ER to present at the cell surface (7). VitBAg that cannot form a Schiff base (yellow) does not release MR1 from the ER, but likely loads onto MR1 at the cell surface or in endosomes (8).

microbial ligands [1,2,10,15••,22•]. There are two ways ligands could effect this change in MR1 localization. First, they might, alone or in combination with other microbial products, trigger a signalling cascade that results in the release of MR1 from its ER-retention mechanism. This would enable MR1 to traffic to the cell surface or endosomal compartments, where it might bind the ligands for MAIT cell stimulation. The second way ligands could cause MR1 to leave the ER is by themselves accessing the ER, where their binding to empty MR1 might cause a change in conformation to the 'closed' state, which in turn would release the MR1–ligand complexes from the retention mechanism. Two lines of evidence support the second model. First, synthetic VitBAgs are not known to trigger cell signalling, and analogs that cannot bind MR1 do not cause ER egress of empty MR1. Second, if cells are incubated with VitBAg and Brefeldin A to block MR1 exit from the ER, MR1–VitBAg complexes accumulate in this location [15••,22•], indicating the ER rather than the cell surface or endosomal compartments is the site of complex formation. The critical step that causes complete folding and release from the ER is the neutralisation of the positively charged lysine 43 residue (K43) in the MR1 ligand binding site. This occurs by formation of a Schiff-base-bond with VitBAg ligands, because mutating this site to a neutral alanine allows release of the mutant MR1 molecules while mutating it to arginine, which retains a positive charge but cannot be neutralised by Schiff base bond formation, cannot exit the ER even in the presence of ligands [15••].

To summarize, most MR1 molecules remain inside the ER in an immature state, ready to capture short-lived metabolite ligands, so the rather unexpected function of these molecules is as an ER-resident sensor of microbial metabolites (Figure 1).

Endosomal Ag loading and non-Schiff base ligands

While it is clear that the loading of extracellular VitBAg occurs efficiently in the ER [3,15••,22•,23], there is some debate regarding the site of Ag loading during infections by *intracellular* microbes (those that live within endocytic compartments or the cytosol). MR1 presentation of Ag

from intracellular *Salmonella* was largely inhibited by brefeldin A treatment [15••], and presentation of *E. coli* Ag was only inhibited by ER/Golgi inhibitors but not lysosomal inhibitors [24•], evidence of intra-ER binding. On the other hand, Harriff *et al.* [22•] suggested there may be distinct routes for presentation of extracellular and intracellular-derived ligand. They observed that knocking down VAMP4 and Rab6 inhibited MR1 presentation of intracellular *M. tuberculosis*-derived Ag without affecting presentation of extracellular 6-FP. The authors proposed that VAMP4 and Rab6 operate in endosomes and specific impairment of intracellular antigen presentation in their absence was indicative of endosomal loading. However, studies on VAMP4 and Rab6 indicate they are involved in ER-to-Golgi transport and/or organisation of the latter [25,26], so while endosomal loading of *M. tuberculosis*-derived Ag to MR1 may happen in endosomes, it cannot be discarded that it can also — or only — take place in the ER. We have observed that MR1 can load ligands in endosomal compartments, but this requires first to accumulate on the plasma membrane MR1-ligand complexes produced in the ER, which could then be transported to endosomes, capture a new ligand, and recycle back to the surface [15••]. This pathway might be particularly relevant for presentation of non-Schiff base-forming metabolites, such as the ribityl lumazines. Since these compounds do not neutralise the MR1 lysine 43 charge, they fail to release MR1 from the ER to the surface, barely upregulate surface MR1 and are very weak MAIT cell agonists [1,27•]. However they might bind to recycling MR1 in endosomes, provided a Schiff base-forming ligand is also present to produce the cohort of surface MR1 molecules required for this pathway (Figure 1). This mechanism might also enable presentation of intracellular microbe Ags that cannot access the ER. Recently another mechanism has been proposed that may favour endosomal MR1 loading. TLR2 and TLR9 agonists increase MR1 surface expression in the absence of ligand in the macrophage line THP1 [28•] and B cells [24•] respectively, and inflammatory cytokines do so in a β-islet cell line [29]. The underlying mechanism is unclear; it may involve increased MR1 transcription, translation, enhancement of empty MR1 stability, synthesis of a yet-unknown endogenous ligand, or increased recruitment to the cell surface or the endocytic route. Additional work is required to obtain a full picture of the relative contribution of MR1 loading outside the ER to MAIT cell-mediated immune responses.

The growing family of MR1 metabolite ligands: towards novel therapeutics?

Structural studies have shown that the MR1 Ag-binding cleft is larger than the space occupied by the currently known ligands, and it exhibits structural flexibility suggesting it might accommodate a wider range of metabolites than the vitamin B2 and B9-derived compounds described so far [8]. Indeed, novel MR1 ligands that either induce or block MAIT cell activation have been recently synthesised or identified. In this section we provide an update on this exciting and fast-moving field that may herald the development MAIT cell-targeted immuno-therapeutics.

In an effort to overcome the relative instability of the canonical MAIT-activating ligand 5-OP-RU, Mak *et al.* [12••] synthesised a highly stable analogue of 5-OP-RU which could activate MAIT cells *in vivo*. Variants of 6-FP capable of effectively blocking MAIT activation have also been synthesised [7•]. An *in silico* screen resulted in the discovery of a range of small organic molecules that bind to MR1, with some candidates able to activate MAIT cells. Notably, one compound, 3-formylsalicylic acid, could block MAIT cell accumulation induced by 5-OP-RU stimulation *in vivo* [27•]. These studies illustrate how rational design of MR1 ligands may result in ligands with improved characteristics that might have therapeutic potential.

Several groups are also attempting to discover novel naturally-occurring MAIT-activating ligands, even unrelated to the riboflavin synthesis pathway. Meermeier *et al.* identified an atypical MAIT cell clone that recognised a compound in supernatant from *Streptococcus pyogenes* in an MR1-dependent manner [30]. This bacterium does not synthesise riboflavin, suggesting it produces a novel but yet unidentified type of MR1 ligand.

A third line of research towards identification of novel MR1 ligands is looking for endogenous (mammalian) compounds. Indirect evidence for the existence of such ligands comes from the fact that MAIT cells are selected during development in the thymus in a MR1-dependent fashion. Selection still occurs in germ-free mice, albeit at reduced numbers [31], indicating that the MR1 molecules recognised by the developing MAIT cells are either empty or bound to an unknown endogenous ligand. If the latter, this appears to be produced only in the thymus, because it is known that expansion of thymic MAIT emigrants in the periphery is strongly dependent on the presence of microbes [6,32]. Two reports further suggest the existence of endogenous MR1 ligands, in this case produced by tumour cells [33•,34••]. Lepore *et al.* [34••] found MR1-restricted T cells in human donors which proliferated upon co-culture with a cell line overexpressing MR1, without microbial ligand present. The putative ligands recognised by these T cells were present in hydrophilic fractions from the THP1 cell line, and were recognised on murine breast tumours in a MR1-restricted fashion. Additional data suggested these ligands, of yet-unknown structure, did not form Schiff-base bonds with MR1. Together these findings suggest the immune surveillance role of the MR1–MAIT cell axis may extend beyond microbe detection to monitoring tumour growth. The repertoire

of MR1 ligands is thus likely greater than initially appreciated.

Future directions and concluding remarks

MR1 has been adapted by the mammalian immune system to sense foreign microbes by their metabolic signature, a unique feature among Ag-presenting molecules. Understanding the cell biology of how MR1 accomplishes this task is an ongoing area of investigation, with some discrepancies related to the role of endosomal loading that further research should resolve. How VitBAgs and perhaps other ligands access the ER for MR1 loading remains a mystery that will require the application of innovative tools and experimental approaches to unravel. Finally, the recent discovery of novel synthetic and natural ligands, both microbial and endogenous, indicates that the MR1–MAIT cell recognition system plays more functions than remain largely unknown. The discovery of these functions and of the molecular players that underpin them will undoubtedly provide new surprises and therapeutic opportunities in this fast-moving area of research.

Conflict of interest statement

Nothing declared.

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