The burgeoning role of MR1-restricted T-cells in infection, cancer and autoimmune disease

Michael D. Crowther[1,i] and Andrew K. Sewell[1,2]

1. Division of Infection and Immunity, Cardiff University School of Medicine, Henry Wellcome Building, University Hospital Wales, Cardiff CF14 4XN, Wales, UK

2. Systems Immunity Research Institute, Cardiff University, Cardiff CF10 3AT, Wales, UK

i. Present address: Department of Oncology, National Center for Cancer Immune Therapy (CCIT-DK), Copenhagen University Hospital Herlev, 2730 Herlev, Denmark

Corresponding author: AKS sewellak@cardiff.ac.uk

HIGHLIGHTS

- *MR1 is an evolutionarily conserved antigen presentation platform*
- *MR1 presents endogenous and bacterial metabolite ligands to T-cells*
- *Recent data shows MR1-restricted T-cells may play important roles in cancer*
- *The conserved nature of MR1 may allow pan-population therapies for many diseases*

ABSTRACT (105 words)
MR1 is a ubiquitously-expressed, monomorphic antigen presenting molecule that has been largely preserved throughout mammalian evolution. The primary role of MR1 is to present conserved microbial metabolites to highly abundant mucosal-associated invariant T (MAIT) cells. The role of MAIT cells and other MR1-restricted T cells (MR1T) has been recently extended to immunomodulation during cancer. MR1Ts have also been implicated in autoimmune disease. The highly conserved nature of MR1 across the human population is in stark contrast to the MHC molecules recognised by conventional $\alpha\beta$ T-cells, therefore MR1Ts may form fertile ground for the development of pan-population T-cell immunotherapeutics for a wide range of important morbidities.

INTRODUCTION

Conventional αβ T-cells orchestrate immunity by recognising short foreign peptides bound to molecular presentation platforms called major histocompatibility complex (MHC) molecules. This ingenious system enables T-cells to interrogate the proteome to identify and eliminate causes of proteomic perturbation such as infection and cancerous transformation. More recent discoveries have identified that T-cell antigens extend beyond the convention of MHC-bound peptides to include 'unconventional' lipid and metabolite antigens presented by CD1 and MHC-related molecule 1 (MR1) respectively. MHC class I (MHC-I), the CD1 family of molecules (CD1a-d in human) and MR1 are similarly comprised of a small β2-microglobulin chain assembled with a larger α chain with the α1 and α2 domains forming a membrane-distal antigen-binding cleft (**Figure 1**). These analogous structures allow T-cells to utilise their hypervariable T-cell receptor (TCR) to inspect the internal proteome, lipidome and metabolome from the cell surface and eradicate cells containing potentially dangerous anomalies. Here we focus on the expanding functions of human T-cells that recognise their target antigens in the context of MR1 (so-called "MR1-restricted" T-cells or MR1T).

MR1

The peptide antigens recognised by conventional MHC-I-restricted T-cells vary widely between different pathogens, even within species [1]. This antigen diversity is reflected by the many thousands of human genes encoding different MHC alleles. In contrast, MR1 is monomorphic and exhibits strong conservation throughout mammalian evolution [2]. MR1 is ubiquitously expressed by almost all nucleated human cells [3] but unlike conventional MHC-I molecules, it is mostly retained the endoplasmic reticulum until binding of an antigen results in transient expression at the cell surface [4,5]. Consequently, basal expression of MR1 at the cell surface is generally below the limits of detection on most cells but increases upon cellular infection [6].

MR1 ligands

A landmark study in 2014 showed that MR1 could bind to antigens derived from the Vitamin B2 (riboflavin) metabolic pathway [7]••. This essential vitamin is synthesised by many bacteria, yeast and plants but not by animals which must acquire it from their diet. Thus, the presence of riboflavin biosynthesis within a mammalian cell serves as a universal signature of

the presence of microbes, either through infection or from commensal origins. MR1 captures pyridine intermediates in riboflavin biosynthesis after their reaction with small molecules derived from glycolysis [8]. T-cell reactivity is dependent on production of the riboflavin intermediate 5-amino-6-d-ribitylaminouracil (5-A-RU) and is unaffected by mutations in downstream enzymes [9]. MR1 cannot be refolded with 5-A-RU but instead forms potent T-cell antigens through nonenzymatic reactions with glyoxal and methylglyoxal [7]••. The structure of two such short-lived antigens 5-(2-oxoethylideneamino)-6-D-ribitylaminouracil (5-OE-RU) and 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) complexed with MR1 has been solved [7]••. More recent liquid chromatography-mass spectrometry studies have revealed that MR1 can bind a wide array of structurally-distinct, bacterially-derived ligands [10]•. Thus, the conserved MR1 molecule appears to act as a sensor for microbial metabolites that is capable of presenting a wide array of different ligands in much the same way that individual MHC-I molecules can present large numbers of different peptides from proteomic anomalies [1] and the CD1 family presents different types of lipids [11,12]. The nature of most MR1 ligands remains undiscovered but the known metabolite ligands occupy a small fraction of the MR1 binding groove compared to peptides in MHC and lipids in CD1 (**Figure 1**), so it remains possible that MR1 might present larger molecular structures that could possibly extend beyond metabolites. Indeed, recent *in silico* predictions identified that aspirin analogues such as 3- and 5-formylsalicylic acids, 2, 4-diamino-6-formylpteridine (2,4-DA-6-FP) derived from methotrexate, and the anti-inflammatory drug diclofenac can bind to MR1 and modulate MAIT cell activity [13] . Studies to date have highlighted the particular importance of the positively charged lysine residue situated on the floor of the MR1 antigen-binding cleft at position 43.

The role of lysine 43

Lysine 43 plays a prominent role in retaining MR1 in the ER prior to ligand capture and cell surface trafficking [5]. Furthermore, the known T-cell ligands, 5-OP-RU and 5-OE-RU, are short-lived pyrimidine adducts that are stabilized through the formation of a Schiff base with lysine 43 (**Figure 2**) which in turn neutralizes the charge on this residue and allows presentation of the MR1-bound cargo at the cell surface (**Figure 3**) [5].

Discovery of MR1T cells

A small fraction (~1%) of peripheral T-cells do not express the CD4 or CD8 coreceptors that govern recognition of MHC class II and class I respectively [14]. These 'double negative' T-cells are thought to be enriched for cells that recognise non-MHC ligands [15]. Analysis of this MHC-agnostic T-cell population from five human donors in 1993 revealed that they express a very diminished and skewed TCR repertoire [15]; the most striking feature of which was a 'public' TRAV1.2 invariant TCR chain estimated to comprise between 3-10% of total TRAV mRNA in peripheral blood lymphocytes across donors. Cells with semi-invariant TRAV1.2-TRAJ33/20/12 TCRs were subsequently found to be highly enriched in the intestinal lamina propria resulting in them being called mucosal-associated invariant T (MAIT) cells [16]. Thanks to the development of soluble fluorochrome-conjugated MR1 multimers, we now know that MAIT cells can also express CD8 (mostly as a CD8$\alpha\alpha$ homodimer) and CD4 resulting in them being far more numerous than originally appreciated [17]. Indeed, MAITs represent between 0.1-10% of the peripheral T-cell pool, and up to 40% of T-cells in mucosal tissues and the liver [18] ensuring that they are the best-studied MR1Ts.

MAIT cells

MAIT cells are generally defined by their expression of a TCRα chain comprised of either TRAV1.2-TRAJ33, TRAV1.2-TRAJ12 or TRAV1.2-TRAJ20. This semi-invariant TCRα chain pairs with a limited array of TCRβ chains including TRBV20 and TRBV6 [19,20]. MAIT cells and MR1 also coevolved, like most invariant cells and their corresponding antigen presenting molecule [21]. MAIT cells are further defined by their high expression of the PLZF transcription factor, which is also expressed in MAIT-like T-cells (or non-classical MAIT cells) – those with similar reactivity to MAIT cells but without the canonical TCR [22]. MAIT cells possess a wide range of effector functions and can directly lyse cells expressing their target antigen through perforin and granzymes, in addition to secretion of a range of cytokines such as IFN-γ, TNF and IL-17A (**Figure 4**) [23]. The array of TCRs expressed by MAITs suggests that they might recognise a range of distinct metabolite antigens with different ligands favouring different TCRβ chains [24,25]. The precise nature of many of these antigens remains unknown but the discovery that MAIT cells could respond to most, but not all, bacteria and yeast [26] initiated the hunt for microbial ligands in earnest and resulted in discovery of the ribityl tail-possessing [27] agonist rRL-6-CH_2OH [28] and the most potent MAIT ligands 5-OP-RU and 5-OE-RU as

described above [7] . MAIT cells are notable in their potency for antigen and exhibit TCR affinities comparable with the very best antiviral conventional T-cells (K_D ~ 1-10 μM) [29]. Contrastingly, MAIT cells are inhibited by the photodegradation product of folic acid 6-formylpterin (6-FP), and can display fine antigen specificity even amongst antigens containing ribityl tails [27]•. A new class of MAIT-inhibiting molecules has also been identified, that function to sequester MR1 inside the endoplasmic reticulum, preventing antigen presentation to MAIT cells [30]. MAIT cells are also notable for their high expression of the NK cell marker CD161 in addition to high levels of the receptors for IL-7, IL-12, IL-15 and IL-18 which enable activation by cytokines without direct TCR stimulation [26], as in the case of MAIT responses to viruses [31].

MR1Ts in infection

The ability of MAIT cells to secrete such diverse cytokines and directly kill infected cells makes them an important subset of T-cells. In mice, where the antigen binding domain of MR1 is >90% identical to human [32], MR1 is critical for immunity to the riboflavin-producing *Francisella tularensis* [33] and *Klebsiella Pneumoniae* [34]. MAIT cell deficient mice are also defective in immunity to *Mycobacterium bovis* bacillus Calmette–Guérin BCG [35,36]. Further studies in mice have also shown MAIT cells regulate the microflora, with direct MAIT cell recognition of microbial antigens promoting tissue repair and therefore reducing the risk of infection [37]••. Furthermore, MAIT cell abundancies are heavily influenced by the microflora, suggestive of a symbiotic relationship between MAIT cells and the microbiota [38]. The most direct evidence of the importance of MR1 during human bacterial infection came with discovery that an intronic single polynucleotide polymorphism in MR1 is associated with reduced mRNA expression and susceptibility to tuberculosis [39].

Noncanonical MAITs

The MR1 ligandome is diverse and present in bacteria such as *Streptococcus pyogenes* that lack the riboflavin biosynthetic pathway and extends to non-riboflavin-derived ligands from *Escherichia coli* and *Mycobacterium smegmatis* [10,40]. This greater range of ligands is mirrored by the TCRs that respond to them which can be TRAV1.2-negative [40] and encompass a range of distinct TCRβ chains that contribute to ligand discrimination [41]. Consequently, the range of MR1T/MAIT cell types and the MR1-bound microbial ligands they

respond to is almost certainly greater than currently known. Indeed, such MR1-restricted but phenotypically ($PLZF^-$, $TRAV1.2^-$) and functionally distinct from MAIT cells have been classified as 'atypical MR1-restricted T-cells' [22]. As such cells do not possess evolutionarily conserved features, their biological significance remains unclear and warrants further investigation. Despite this, very rare cells could be important for the discovery of TCRs with therapeutic potential and for illuminating the path towards important differences in cellular metabolism between healthy cells and cancer cells [42]••.

Self-reactive MR1Ts

Lepore and colleagues extended the family of human MR1Ts by priming T-cells with A375 cells over expressing an MR1-β2m fusion gene construct in the absence of exogenously applied antigens [43]•. All seven reactive clones tested were inhibited by addition of 6-FP to A375 cells overexpressing wildtype MR1, but still recognised targets overexpressing K43A MR1 in the presence of high levels of 6-FP demonstrating that these T-cells, which expressed a wide variety of different TCRs, likely recognise endogenous antigen(s) that is being displaced by 6-FP. The authors concluded that these new T-cells were stimulated by ligands that do not require the formation of a Schiff base with MR1 via lysine 43. Importantly, this study found that two of the clones could recognise antigen presenting cells pulsed with different hydrophilic fractions purified from THP1 cells or the mouse breast cancer line EMT6, demonstrating that the unknown antigens were: (i) varied (as the different clones responded to different fractions); (ii) stable within cell lysates; and, (iii) conserved between mammalian species. These new T-cells were found to exhibit MR1-dependent T helper-like capacities and frequently recognised monocyte-derived dendritic cells (moDC). The authors conclude that this novel population of MR1Ts "*might drive inflammatory responses, support B cell function, mediate DC licencing promote tissue remodelling and contribute to mucosal homeostasis*" [43]•.

Cancer-specific MR1Ts

We recently added to the MR1T family by describing a T-cell exhibiting MR1-dependent recognition of many human tumour types and primary tumours while remaining inert to healthy cells (including MoDC) [42]••. These cells exhibited potent cytotoxic activity and cytokine secretion at effector to target ratios below that we have ever seen for conventional

MHC-restricted anticancer T-cells. Activation was inhibited by 6-FP or K43A mutation of MR1 suggesting that the TCR ligand formed a covalent link to MR1 analogous to the known bacterial ligands [44]. Our unpublished observations show that these cells cannot be stimulated with lysates of the best cancer targets, indicating that the antigen might be labile. We also found other TCRs from different donors that behave like MC.7.G5 T-cell despite expressing different TCRs. While we were able to isolate the relatively common (>1:5000 blood T-cells) self-reactive MR1Ts described by Lepore and colleagues [42]•, we found MC.7.G5-like cells to be over 100 times rarer. MR1 expression on cancers is not a new concept [45], with MAIT cells appearing to have an ambiguous role in a wide range of cancers from inducing tumour cell growth arrest in colorectal cancer *in vitro* experiments [46], promoting tumour metastases in lung mouse models [47], and being able to kill myeloma cells that were pulsed with the activatory MAIT cell ligand 5-OP-RU [48]. This latter point is particularly interesting due to recent evidence demonstrating that different tumour types have distinct microbiome compositions [49]••.

MR1Ts in autoimmune disease

MR1Ts are now thought to play other important immune roles. The discovery that some MR1Ts can recognise endogenous ligands [42]• or MR1 independent of ligand [50] opens the possibility that they are relevant to autoimmune disease. MAIT cells have already been implicated in inflammatory bowel disease (IBD) due to decreased MAIT cell numbers in IBD flares, suggestive of inflammation site recruitment [51]. Similarly, extravasation of MAIT cells to myelin sites occurs during periods of myelin degeneration in patients with Multiple Sclerosis, however their role remains unclear [52,53]. Cytokine-mediated activation of MAITs has been found to promote inflammation and exacerbate disease in murine models of arthritis [54], despite appearing to help control anti-islet autoimmune responses in Type 1 diabetes [55]. Further work will be required to determine whether the high frequency of MAIT cells and their ability to activate in response to cytokines means that MAIT cells add to an already bad situation or whether MR1-restricted TCR-mediated signalling is involved at disease sites.

CONCLUSIONS

The strong conservation of MR1 and corresponding bacteria-reactive invariant T-cell subset throughout most of mammalian evolution suggests that MR1Ts play an important role. MR1 can bind to a large number of distinct bacterial [56] and endogenous ligands but the precise molecular nature of these molecules remains unknown. It is also becoming apparent that some MR1Ts are capable of distinguishing cancer cells from healthy cells and may be involved in autoimmune disease. Dissection of how various MR1T subpopulations contribute to all these roles will be advanced with the identification of the relevant MR1-bound antigens - a likely major focus of future MR1T research. T-cell therapies, especially for cancer, have exploded over the last decade with chimeric antigen receptor (CAR)-T therapy having demonstrated remarkable success for treatment of some forms of leukaemia [57]. This success has seen renewed interest in TCR-T therapy for cancer and other diseases. The biggest drawback of the conventional TCR is its restriction to highly variable MHC presentation platform which means even targeting through the most frequent HLA in the population is only applicable in a minority of patients. The monomorphic nature of MR1 and its conservation within the most commonly used animal models of disease ensures that MR1Ts, their TCRs and MR1-bound ligands may represent the richest ore for future extraction of important pan-population immunotherapeutics [58].

Declaration of Interest: MDC and AKS are beneficiaries of a Cardiff University patent describing the recognition of cancer cells via MR1. AKS is on the scientific advisory board of Enara Bio.

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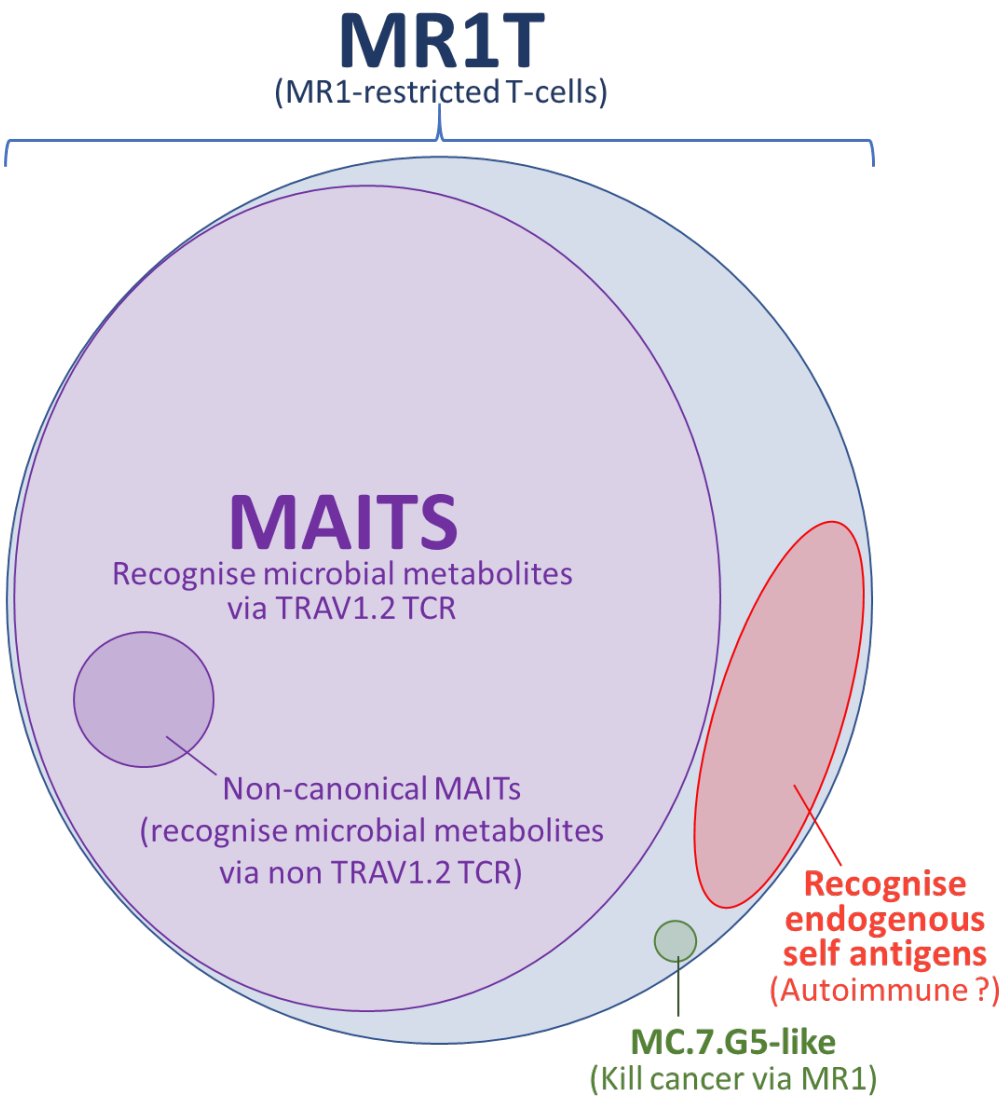

Graphical abstract:

MR1-restricted T-cells (MR1Ts) encompass an ever increasing range of T-cells. MR1T can represent ~10% of total T-cells with the vast majority of these being mucosal-associated invariant T (MAIT) cells which recognise microbial metabolites, canonically intermediates in riboflavin biosynthesis via a TRAV1.2 semi-invariant T-cell receptor, although noncanonical MAITs have been described. Furthermore, MR1T cells have been identified that recognise self-antigens presented by monocyte-derived DCs, and MC-7.G5-like cells that recognise cancer.

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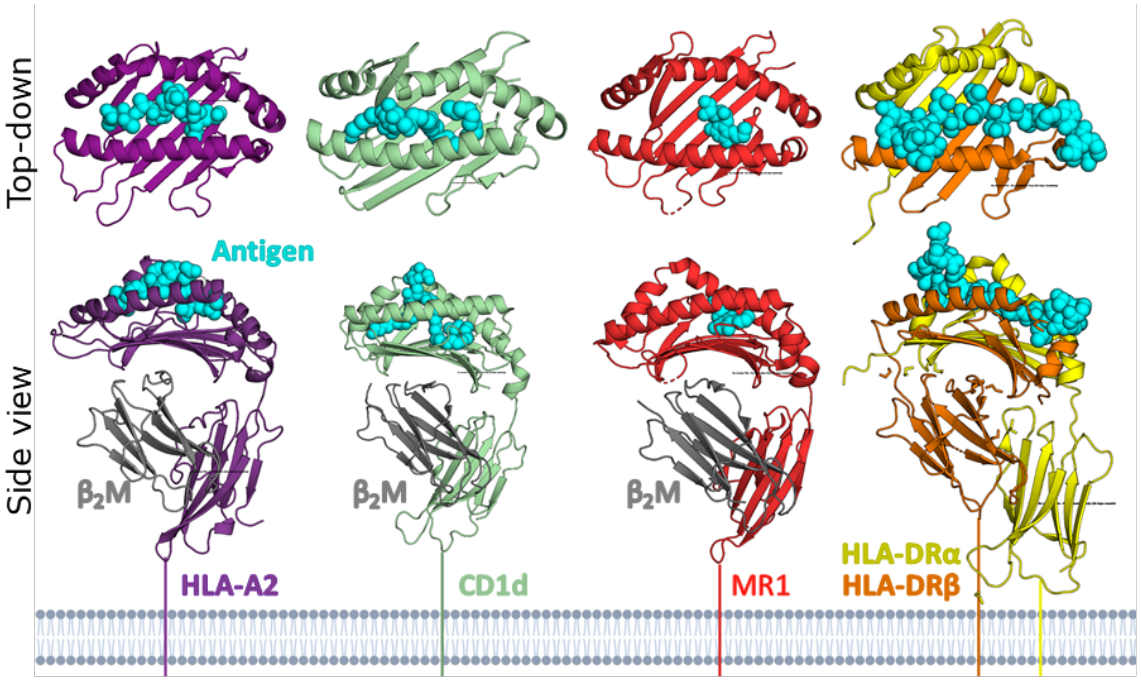

Figure 1. Protein structures of antigen presenting molecules. Antigen binding domains of HLA-A2 complexed with SLYNTIATL peptide, CD1d with α-galactosylceramide, MR1 with 5-OP-RU and HLA-DR with ARRPPLAELAALNLSGSRL peptide. β2-microglobulin is common to HLA-A2, CD1d and MR1.

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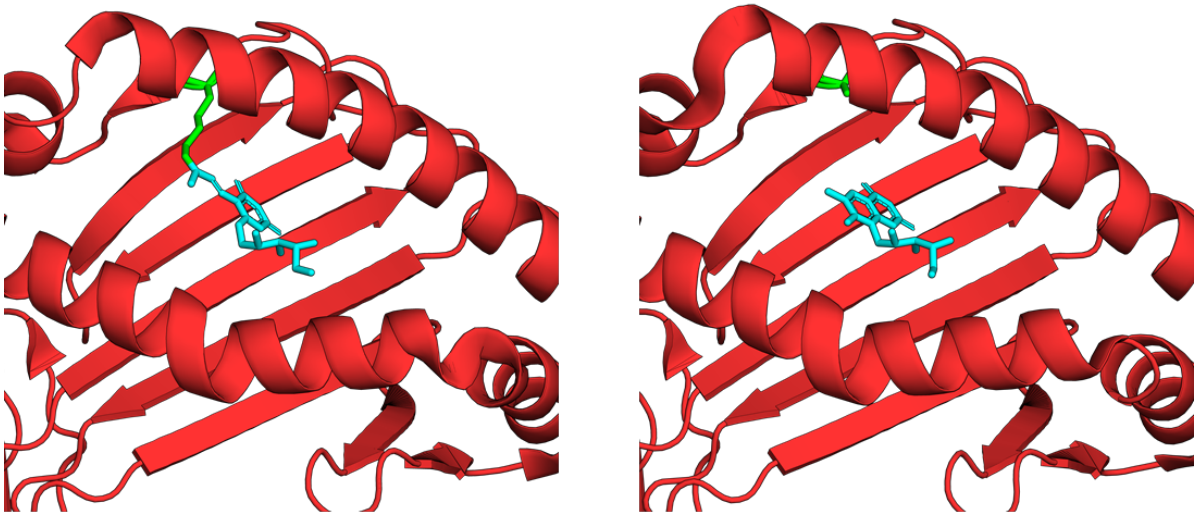

Figure 2. MR1 ligands in wildtype or mutated MR1. MR1 ligands (turqouise) bind by Schiff base formation with K43 (green). Mutating K43 to A (right) neutralises the positive charge and results in stabilised MR1 enabling surface expression. MR1-K43A can be either 'empty' with no ligand, or it can present ligands to T-cells without covalent bonding.

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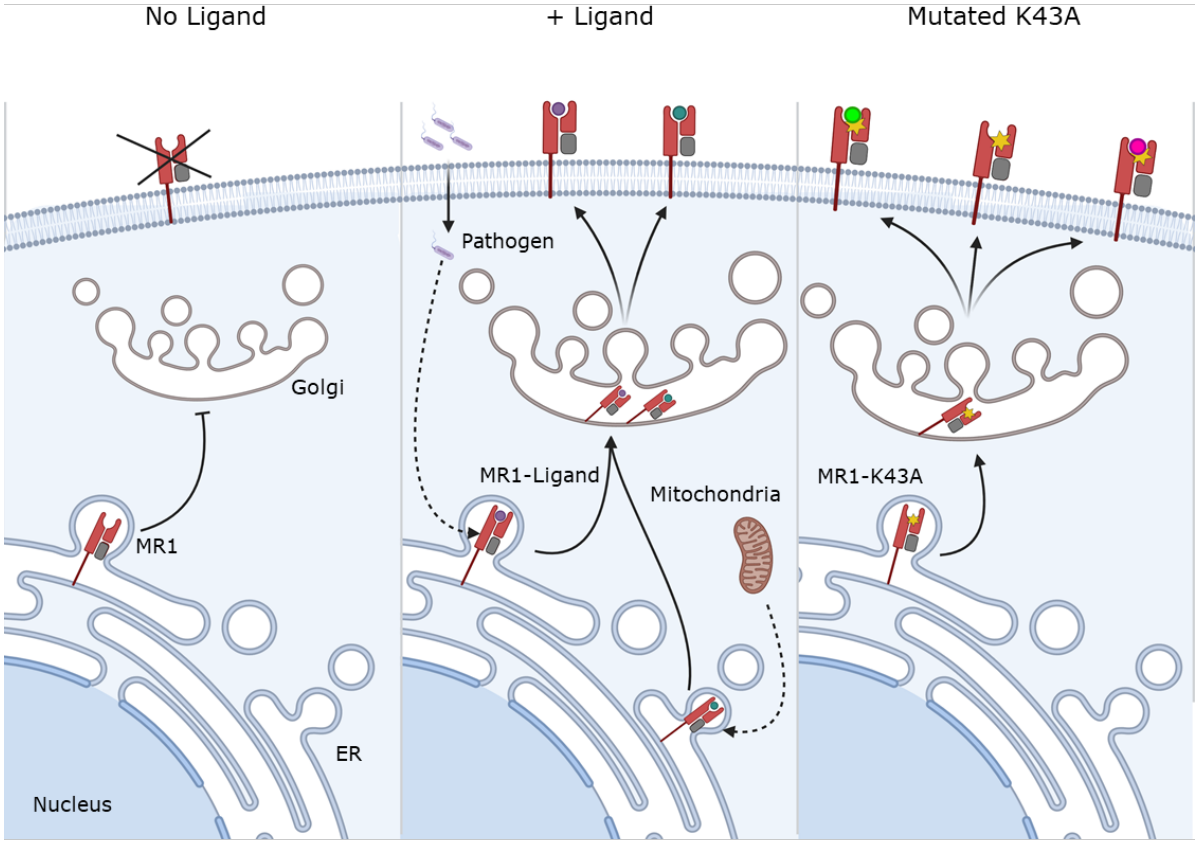

Figure 3. MR1 ligand loading occurs in the endoplasmic reticulum (ER) to facilitate surface expression. With no ligand present, MR1 is retained in the ER. Ligand sources can be from metabolic derivatives from somatic sources or infection, resulting in MR1-ligand surface expression. Mutation of MR1 antigen binding domain at position 43 (e.g. K43A) results in stabilisation of MR1 enabling surface expression without ligand. Non-covalent ligand binding can still occur.

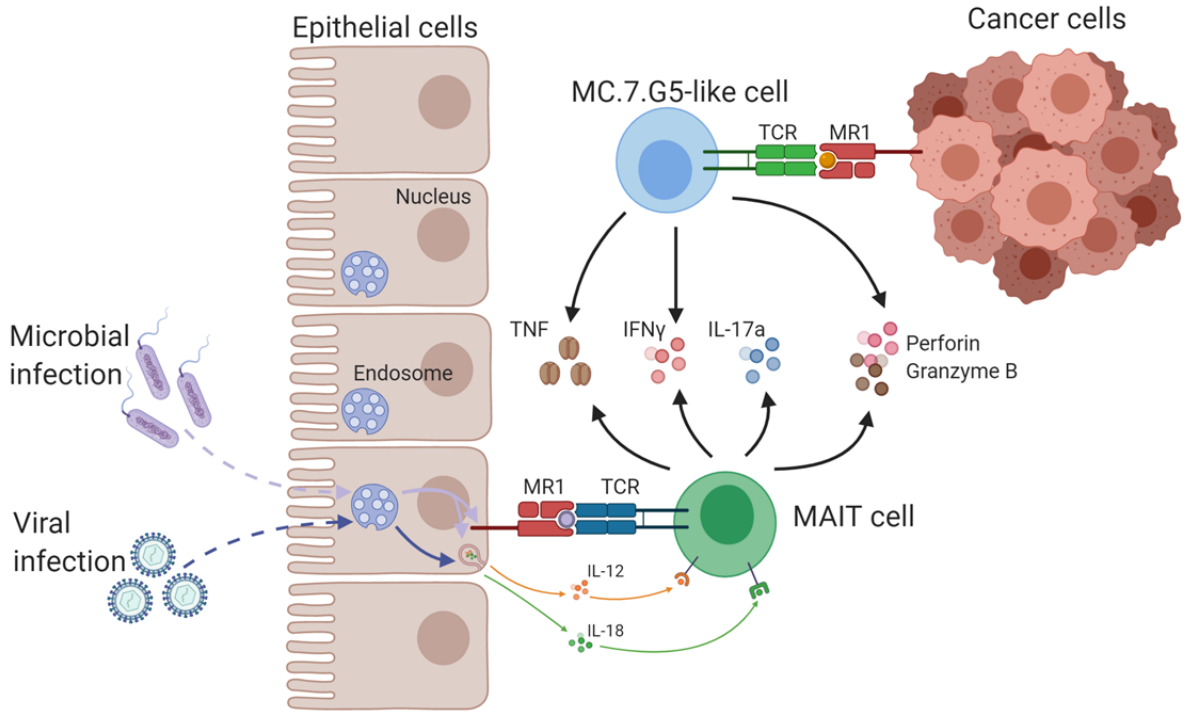

Figure 4. Distinct modes of recognition between MAIT cells and MC.7.G5-like cells. MAIT cells are master regulators of the microbiome, and have been shown to secrete cytokines and directly kill cells infected with microbes that produce riboflavin metabolites. MC.7.G5-like cells do not recognise microbes, but instead recognise cancer cells, while secreting a similar range of cytokines with specific killing of antigen-positive cancer cells.

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