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Mucosal-Associated Invariant T cell receptor recognition of small molecules presented by MR1

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Abstract

The major histocompatibility complex (MHC) class-I related molecule MR1 is a monomorphic and evolutionary conserved antigen (Ag)-presenting molecule that shares the overall architecture of MHC-I and CD1 proteins. However, in contrast to MHC-I and the CD1 family that present peptides and lipids, respectively, MR1 specifically presents small organic molecules. During microbial infection of mammalian cells, MR1 captures and presents vitamin B precursors, derived from the microbial biosynthesis of riboflavin, on the surface of antigen presenting cells (APCs). These MR1-Ag complexes are recognized by the mucosal-associated invariant T cell receptor (MAIT TCR), which subsequently leads to MAIT cell activation. Recently, MR1 was shown to trap chemical scaffolds including drug and drug-like molecules. Here, we review this metabolite Ag-presenting molecule and further define the key molecular interactions underlying the recognition and reactivity of MAIT TCRs to MR1 in an Ag-dependent manner.

Introduction

T cell immune surveillance involves the activation of a T cell in response to a specific non-self-antigen (Ag). Upon pivotal recognition by the αβ T cell Ag receptor (TCR) of the Ag-presenting molecules loaded with a given Ag, the TCR transmits signals to the CD3 complex that subsequently prompts an intracellular signaling cascade, culminating in an effective immune response[1, 2]. Each α- and β-TCR chain consists of a variable (V) and a constant (C) domain to form a paired αβ TCR heterodimer. The Complementarity Determining Regions (CDR) of the α- and β-variable domains are responsible for driving the molecular recognition of the specific Ags that are presented by the MHC and MHC-like cell surface protein families[3, 4].

There exist two classes of highly polymorphic MHC molecules, namely, the MHC class I (MHC-I) and class II (MHC-II), that present peptide-based Ags to $CD8^+$ and $CD4^+$ T cells, respectively. The MHC-I molecule is comprised of a heavy chain consisting of three domains (α1, α2 and α3) and β2-microglobulin (β2m) (Figure 1a). The MHC-I binds antigenic peptides in the peptide-binding groove formed by the α1- and α2-helices. The overall architecture of the MHC-II molecule is comprised of α- and β-chains whereby each possess two distinct domains (α1/α2 and β1/β2,

respectively) that capture the peptide Ags in the peptide-binding groove (Figure 1a & b). MHC-like proteins are monomorphic molecules that share the same overall architecture with MHC-I but have evolved to form Ag binding grooves that can present discrete non-peptide classes of ligands to specific T cell subsets (Figure 1c & d)[5, 6]. For example, the CD1 family (CD1a, CD1b, CD1c and CD1d) presents both foreign and self-lipids to T cells that include the Natural Killer T cells (NKT) and the germline-encoded mycolyl lipid reactive T (GEM) cells[7-10]. Lastly, the MHC class-I related molecule (MR1) presents small molecule metabolites to mucosal-associated invariant T (MAIT) cells[11-18].

MAIT cells are the most abundant innate-like T cell population in humans, and are evolutionary conserved in many other (but not all) mammalian species including mice and cattle[9, 19-24]. A key feature of MAIT cells is that they typically express a TCR with a relatively fixed α-chain (Vα7.2-Jα33, Vα7.2-Jα12, or Vα7.2-Jα20 in humans; and Vα19-Jα33 in mice) that is restricted to MR1[9, 20]. MAIT cell activation can be induced in TCR-dependent and TCR-independent manners. During bacterial infections, derivatives of the riboflavin biosynthesis pathway are captured by MR1 and presented on the surface of APCs, subsequently leading to the activation of MAIT cells via the MAIT TCR-MR1 axis. Thus, MAIT cells are considered to play a protective role in anti-bacterial immunity[12-15]. In contrast, during viral infection (e.g. dengue, hepatitis C and influenza viruses), infected cells release IL-18, IL-12 and IFNα, which can activate MAIT cells in a MR1-independent manner[25-27]. Upon activation, MAIT cells rapidly produce inflammatory cytokines in Th1/Th17-like responses and may also express granzyme B and perforin, killing the infected cells[25, 28-31]. This review will discuss the key molecular interactions underlying MAIT TCR recognition of MR1-restricted metabolites and highlights the uniqueness of this metabolite presentation axis compared to other Ag presentation systems.

MHC and recognition by the αβ TCR

Many structural studies have investigated the molecular interactions underpinning the recognition of Ag-presenting molecules by TCRs (recently reviewed[2, 6, 32]). Typically, the αβ TCR docks atop the MHC molecule, enabling the TCR α- and β-chain CDRs (CDR1, 2 and 3 loops) to interact with the antigen/MHC cleft (Figure 1). The MHC-II binding cleft is more open-ended at the N- and C-termini in comparison to the more confined cleft of MHC-I. Accordingly, MHC-II generally binds longer peptides (>11 amino acids) via a series of binding pockets (P1-P9), while MHC-I typically binds shorter peptides (8-10 amino acids) via the A-F pockets [33] (Figure 1a and b). Most (but not

all[34, 35]) of TCR-MHC-peptide ternary complexes share a common docking topology where the Vα and Vβ domains of the TCR reside over the α2- and α1-helices of the MHC-I molecule, respectively. Similarly, the TCR Vα and Vβ chains reside above the α-helices of the β- and α-chains of the MHC-II molecule in the TCR-MHC-II-peptide complexes, respectively (Figure 1a and b).

In the CD1 family, each member exhibits a characteristic Ag-binding cleft architecture that comprises a number of pockets (termed A′, C′, F′, and T′) and accessory portals (C′ and D′/E′ portals) that can specifically accommodate distinct self- and/or foreign lipids (see review[5, 36 6]). For example, CD1b possesses a large and deep Ag binding-cleft consisting of four pockets (A′, C′, F′, and T′). The hydrocarbon chains of the amphipathic lipids sit in the CD1b cleft and their hydrophilic head groups are protruding out the F′-pocket exposed for TCR recognition [37] (Figure 1c). The TCR-CD1-lipid docking topologies can be distinctive from the TCR-MHC-peptide interactions[5, 38]. For example, the first TCR-CD1d-lipid ternary complex revealed that the NKT TCR bound parallel to the CD1d-Ag binding-cleft [39] contacting both the CD1d and the lipid-based Ag, while in the TCR-CD1a-lipid complex, the TCR recognized CD1a exclusively, thereby breaking the co-recognition paradigm implicit in the TCR-MHC axis[40].

Similar to the MHC-I and CD1 families, MR1 protein contains a heavy chain that is non-covalently bound to β2m and possesses an Ag-binding cleft that is composed of only two distinct pockets (A'- and F'-pockets) (Figure 1d) [41]. The MR1 A'-pocket consists of an 'aromatic cradle' that is delineated by both charged and hydrophobic residues to form the Ag binding-pocket[41]. Notably, the MR1 binding cleft (760 Å^3) is much smaller than that of any of CD1 isoforms. Accordingly, the chemical and structural characteristics of the MR1 pocket are distinct from that of peptide and lipid-based Ag-binding pockets, with the MR1 pocket being ideally shaped to capture small molecule compounds[41, 42]. Similar to the TCR-MHC-I-peptide complex, the MAIT TCR sits approximately centrally in an orthogonal docking mode to the main axis of the MR1-Ag binding cleft (Figure 1d), and the α- and β-chains of the MAIT TCR reside over the α2- and α1-helices of MR1, respectively[42-44].

MR1 presenting vitamin B derivatives

The MR1 protein is ubiquitously expressed in all cells, but is presented on the cell surface at very low or undetectable levels that is strictly dependent on antigen availability[45-47]. In the early 2000s, it was shown that MAIT cells are restricted to MR1 and activated by a broad range of bacteria and yeast[9, 23, 48]. This suggested conserved Ag(s) common to these activating microbes, however, the nature of these Ags remained unknown. Recently, Kjer-Nielsen *et al.*[41] successfully identified the MR1 ligands as small organic compounds, where two classes of vitamin B derived compounds were described to be presented by MR1 (Figure 2a) and activate MAIT cells to varying degrees of potency (see reviews[16, 18, 49]). The first class included 6-formylpterin (6-FP), a photodegradation product of folic acid (vitamin B_9), and its related synthetic compound Acetyl-6-formylpterin (Ac-6-FP)[41, 44]. These two formylpterins have a bicyclic structure with a formyl group; where Ac-6-FP differs from 6-FP by the presence of an acetyl group (Figure 2a). Both compounds can up-regulate MR1 at the cell surface but do not stimulate the majority of MAIT cells. Furthermore, they compete with the activating ligands for binding MR1 and hence are considered as inhibitory ligands to MAIT cell activation. Despite minor chemical difference between 6-FP and Ac-6FP, the latter is a 100-fold more potent inhibitor of MAIT activation in comparison to the former[44, 50].

The other class of MR1 ligands was derived from intermediates of the riboflavin (vitamin B_2) biosynthesis pathway in certain microorganisms, which in contrast to pterin-based ligands, can activate MAIT cells. Importantly, these riboflavin ligands can be synthesized by a wide range of microbes, but not by mammals and accordingly represent a molecular signature of microbial infection[41, 43]. Corbett *et al.* [43] has applied mutagenesis approaches on the *rib*-operon in *Lactobacilus Lactis* and *Salmonella typhimurium* to identify the bacterially produced riboflavin intermediate 5-amino-6-D-ribitylaminouracil (5-A-RU) as an essential intermediate for MAIT cell activation. The microbial 5-A-RU non-enzymatically reacts with ketones/aldehydes, such as glyoxal and methylglyoxal derived from mammalian glycolysis or bacterial metabolism, generating highly potent ribityl pyrimidine ligands[51-53] (Figure 2b). These newly formed and unstable pyrimidines can be captured and stabilized by MR1; they are otherwise rapidly dehydrated and undergo ring closure forming stable ribityl lumazines (Figure 2a and b). Both ribityl pyrimidines and ribityl lumazines can be presented by MR1, however the lumazines are far less potent at activating MAIT cells than pyrmidine Ags[41, 43]. The most potent currently known MAIT cell activators are 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) and 5-(2-oxoethylideneamino)-6-D-ribitylaminouracil (5-OE-RU) [41, 43, 53].

Key molecular interactions underlying MR1-Ag capture and canonical MAIT TCR recognition

The crystal structure of the MR1-6-FP binary complex represented the first three-dimensional structure of MR1 presenting an Ag[41]. Subsequently, a series of high-resolution crystal structures of ternary MAIT TCR-MR1-Ags complexes have been determined for various TCRs recognizing diverse MR1-Ags complexes and thus provide detailed molecular insights into the MR1-Ag recognition by MAIT TCRs. [42-44, 54, 55]. Until now, all identified MR1 restricted Ags are bound within the A′-pocket of MR1. Here, the Ag is captured by a network of interactions with polar (Lys43, Arg9 and Arg94) and aromatic residues (e.g. Tyr7, Tyr62, Trp69, Tyr152 and Trp156) located within the pocket. Importantly, the Lys43 residue at the base of the A′-pocket can form a Schiff-base covalent linkage with the carbonyl group of some Ags (Figure 3a and b). This covalent bond has been shown to be a key feature of MR1 presentation of many Ags, however it doesn't appear to be essential for MR1 ligand binding as the lumazine Ags (*e.g.* RL-6-Me-7-OH) do not form a Schiff base with MR1 (Figure 3c) (and accordingly upregulate MR1 very poorly). The orientation of riboflavin Ags within the MR1 pocket differs from that of folate ligands. Namely, 5-OP-RU and RL-6-Me-7-OH are rotated by ~ 75^{o} with respect to 6-FP. Furthermore, MR1 makes different contacts with the respective ring systems of the ligands (Figure 3a-c). Notably, this tilted conformation of the riboflavins allows the formation of hydrogen bonds between the ribityl tail and two conserved MR1 residues (Arg9 and Arg94), which fix the ribityl tail for MAIT TCR ligation (Figure 3b and c).

The majority of human MAIT cells bear a TCR comprised of an α-chain formed by TRAV1-2 recombined with TRAJ33, TRAJ12, or TRAJ20. These TRAV1-2^{+} α-chains pair with a restricted set of TCR β-chains, biased mainly towards TRBV6-1, TRBV6-4 and TRBV20 gene segments[20, 50]. The semi invariant MAIT TCR (TRAV1-2 / TRAJ33 coupled with TRBV6-1) is docked similarly in an orthogonal mode above the A′-pocket of MR1 for various MR1-vitamin B metabolites complexes. The ribityl tail of the stimulating ligands forms a hydrogen bond with the tyrosine residue (Tyr95) of the CDR3α loop of the TCR whilst 6-FP does not contact the TCR directly. Importantly, this Tyr95α residue is evolutionary conserved among MAIT TCRs, however recent studies describe unconventional MAIT TCRs that do not contain the Tyr95 (see below)[54, 56, 57]. The CDR3β loop of the A-F7 TCR made an additional indirect contact with the pyrimidine adducts

through a water molecule (Figure 3b). This is consistent with mutagenesis studies on MAIT TCRs, which suggested that no discrete Vβ residue is essential for MAIT cells activation [58].

MR1-restricted TCR heterogeneity and ligand recognition

Variations in TRBV gene utilization have been observed in the TCR repertoire. From a structural perspective, pairing of a TRAV1-2 TCR chain with TRBV6-1, TRBV6-4, or TRBV20 TCR chains in humans subtly impacts on MR1-Ag recognition[43, 44, 50]. A comparison of TCRs utilizing these three β-chains shows docking atop MR1-5-OP-RU (Figure 4a), with only little juxtaposition of the TRBV20$^+$ to that of the TRBV6$^+$ chain, resulting in a 17^o degree rotation (Figure 4 b-d). This variation could be assigned to differing Vα-Vβ packing together with varying Vβ-MR1 contacts. Notably, the overall contact area within the TRBV20$^+$ TCR was decreased when compared to the TRBV6-1 and TRBV6-4 TCRs. Whilst the CDR1β loop does not make substantial contacts with MR1 in either TRBV6-1 or TRBV6-4 TCR-MR1-Ag ternary complexes, it marginally interacted with MR1 in TRBV20 TCR-MR1-Ag. The CDR2β loop of the TRBV6-4 chain contributed more to the interactions with MR1 than either of the TRBV6-1 and TRBV20. Notably, the CDR3β loop of all three TCRs still played a major role in contacting MR1-5-OP-RU.

Furthermore, Eckle *et al.* [44] addressed the impact of the use of varied TRAJ regions on the MR1-Ag recognition by MAIT TCRs. Functional, affinity and structural investigations concluded that diverse TRAJ usage (TRAJ33, TRAJ12, or TRAJ 20) disrupted neither the conserved docking mode nor the molecular contacts at the MAIT TCR/MR1-Ag interface, indicating similar functional roles of the TRAJ12, TRAJ20, and TRAJ33$^+$ MAIT TCRs. Regarding the impact of the CDR3β hypervariability, Surface Plasmon Resonance (SPR)-based binding studies of six MAIT TCRs possessing diverse CDR3β loops were shown to exhibit similar affinities towards MR1-5-OP-RU with K_{deq} varying from 2 to 9 μM. Interestingly, a variation in the binding affinity was noticed against MR1-Ac-6-FP whereby one MAIT TCR exhibited higher affinity (K_{deq}~ 35 μM) relative to the other MAIT TCRs (K_{deq}> 200 μM). Structural comparison of these TCR-MR1-Ag complexes revealed a degree of flexibility in the CDR3β loop of the TCRs that subtly altered some contacts with both MR1 and Ag[44]. Thus, the MAIT TCR interactions and consequently its reactivity to MR1 are capable of being fine-tuned in an Ag-dependent manner. This has raised the possibility that the ptertin based inhibitors could be stimulatory to certain MAIT cell populations. Indeed, Gherardin *et al.*[54] recently identified minor subsets of MR1-reactive MAIT TRAV1-2$^+$ TCRs that exhibited

folate-derivative responsiveness and displayed autoreactivity towards MR1-Ag. The crystal structure of a MAIT M33.64 (TRAV1-2/TRBV6-4) TCR-MR1-Ag complex revealed a docking mode akin to folate non-responsive MAIT TCRs. However, the conformational flexibility in the MAIT TCR-MR1 interaction network enabled this folate responsive TCR to recognize both riboflavin and folate-derivative based ligands presented by MR1 (Figure 4e).

Recently, the development of pivotal new technologies, such as MR1-Ag tetramers[50], has greatly assisted to identify diverse folate and riboflavin derivative-reactive MR1-restricted T cells utilizing a diverse atypical (TRAV1-2$^-$) TCR repertoire [54, 57]. Generally, TRAV1-2$^-$ TCRs bind MR1 in an Ag dependent manner akin to TRAV1-2$^+$ TCRs, where some are reactive to folate derived Ags and others respond to riboflavin derived Ags. Interestingly, a subset of MR1-restricted T cells possessing a TRAV12-2$^+$ α-chain recognized *Streptococcus pyogenes* bacterial strain that lack a riboflavin biosynthesis pathway, suggesting the ability for MR1 to present Ags distinct from those currently known[57]. Presently, only one TRAV1-2$^-$ TCR-MR1-5-OP-RU crystal structure has been determined (TRAV36/TRAJ34 coupled with TRBV28) [25]. This TRAV36$^+$ TCR docks more centrally (65^o docking angle) atop the MR1 binding-cleft than TRAV1-2$^+$ TCRs (Figure 4 f and g). The centres of gravity of the TRAV36 TCR α- and β-chains were displaced by approximately 9 Å towards the F′-pocket. Whilst the CDR3α loop played a pivotal role in MR1 recognition of TRAV1-2$^+$ TCRs, here, the CDR1α loop of the TRAV36$^+$ TCR was the key player (Figure 4g). The asparagine residue (Asn29α) from the CDR1α loop represented the contact point between the MAV36 TCR and the Ag by forming an H-bond with the ribityl tail of 5-OP-RU. Furthermore, the CDR2β loop played a greater role in mediating MR1 recognition by the TRAV36$^+$ TCR compared to TRAV1-2$^+$ TCRs. Lastly, the CDR3β loop was distal from the A′-pocket but was nevertheless involved in numerous MR1 contacts. Collectively, MR1-restricted TCR heterogeneity leads to differential MR1 Ag recognition and specificity.

Can MR1 present ligands distinct from vitamin B based derivatives?

To address this key question, Keller *et al.*[55] employed *in silico* docking approaches followed by functional assays and structural investigations to explore whether MR1 could present ligands distinct from riboflavin and folate-based metabolites. A diverse panel of small molecules (Figure 5a), spanning mono- and bicyclic chemical structures were identified, potentially capable of binding to MR1 including small organic molecules, drugs, drug-like molecules and drug metabolites such as

2-hydroxy-5-methoxybenzaldehyde (HMB), salicylates (e.g. 3-formyl-salicylic acid; 3-F-SA), the active component of Sirtinol (2-hydroxy-1-naphthaldehyde; 2-OH-1-NA), a photodegradation product of the chemotherapeutic aminopterin (2,4-diamino-6-formylpteridine; 2,4-DA-6-FP) and the anti-inflammatory drug diclofenac (DCF) or its metabolite, 5-hydroxy-diclofenac (5-OH-DCF). Most of those compounds exhibited non-stimulatory properties, although some diclofenac metabolites were MAIT cell agonists. The ensuing MAIT TCR-MR1-Ag ternary crystal structures showed that the drugs displayed differing orientations and interactions within the A′- pocket of the MR1 cleft (Figure 5b-e). Whilst all the non-stimulatory ligands did not directly bind the MAIT TCR, 5-OH-DCF mediated a hydrogen bond with the CDR3β loop of the TCR (Figure 5f). In conclusion, MR1 is most likely to present a broader range of chemical scaffolds that could modulate MAIT cell function in mammals.

Conclusions

The three-dimensional architecture of the MR1 antigen-binding cleft is distinct from that of classical MHC and CD1 that present peptides and lipids, respectively. Instead, MR1 is ideally suited to bind small organic molecules, including those derived from folic acid and precursors of riboflavin synthesis. Moreover, it has recently been shown that MR1 can present other chemical scaffolds including drugs and drug-like molecules. Direct contact between the MAIT TCR and the compound sequestered within the MR1 cleft appears to correlate with the ability to activate MAIT cells, whereby non-stimulatory ligands fail to make direct contact. It remains to be determined what other naturally occurring ligands can bind MR1 and subsequently modulate MAIT cell activity.

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Conflict of interest

The authors declare no competing financial interests.

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Figure legends

Figure 1. Overview of TCR recognition of peptide, lipid, and metabolite presenting molecules. (a) TCR-HLA-DQ2-gliadin ternary complex (Pdb code: 4OZG)[59], (b) TCR-HLA-B*3508-13mer ternary complex (Pdb code: 2Ak4)[60], (c) TCR-CD1b-GMM ternary complex (Pdb code: 5l2k)[37], and (d) MAIT TCR-5-OP-RU-MR1 ternary complex (Pdb code: 4NQC)[43]. The top panels show a cartoon representation of the crystal structures of the individual ternary complexes. The middle panels show a top view of the Ag binding cleft of the respective MHC and MHC-like molecules. The bottom panels show the molecular surface of MHC-II, MHC-I, CD1b and MR1with their bound antigens. Black-filled circles indicate the center of mass of the Vα and Vβ domains. CDR loops color codes: CDR1α, teal; CDR2α, pink; CDR3α, purple; CDR1β, red; CDR2β, orange; and CDR3β, yellow. Structural illustrations in Figures 1, 3, 4 and 5 were prepared using PyMOL Molecular Graphics System, Version 1.8.6, Schrödinger.

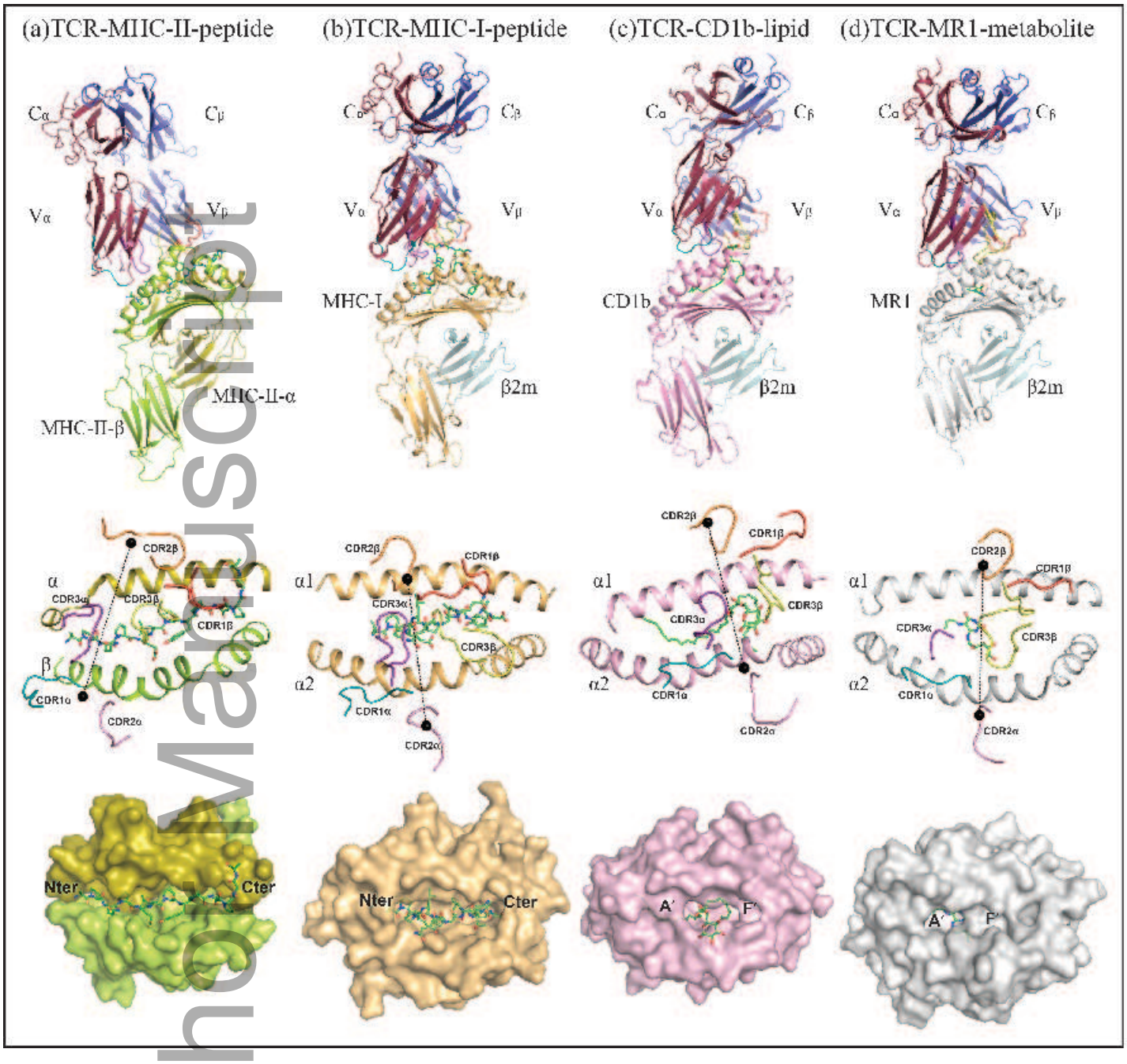
Figure 2. (a) Chemical structures of vitamin B related MR1 Ags including pterins: 6-formyl pterin (6-FP) and acetyl-6-formyl pterin (Ac-6-FP); pyrimidines: 5-(2- oxoethylideneamino)-6-D-ribitylaminouracil (5-OE-RU), 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) and 5-(1-methyl-2-oxopropylideneamino)-6-D- ribitylaminouracil (5-MOP-RU); lumazines : 7-hydroxy-6-methyl-8-D-ribityllumazine (RL-6-Me-7-OH), reduced 6-hydroxymethyl-8-D-ribityllumazine (rRL-6-CH2OH) and 6,7-dimethyl-8-D-ribityllumazine (RL-6,7-diMe). (b) Condensation reactions of bacterially produced riboflavin intermediate 5-amino-6-D-ribitylaminouracil (5-A-RU) with reactive ketones/Aldehydes, which subsequently dehydrated to ribityl lumazine compounds via pyrimidine intermediates.

Figure 3. Presentation of vitamin B derived ligands by MR1. The non-antigenic 6-FP (a), the agonist 5-OP-RU (b), and the weak agonist RL-6-Me-7-OH (c) are shown in the MR1 binding cleft (top panels) and their interactions with the A-F7 MAIT TCR (bottom panels). TCR CDR3α and CDR3β are coloured pink and light blue respectively. Hydrogen bonds are shown as black dashed lines and water molecules as red spheres.

Figure 4. T cell receptor heterogeneity of mucosal-associated invariant T cells. Ternary structure of a typical TRAV1-2^{+} TCR (a) and the footprint of TRAV1-2/TRBV6-1 TCR (b), TRAV1-2/TRBV6-4 TCR (c), TRAV1-2/TRBV20 TCR (d) and autoreactive M33.64 (TRAV1-2/TRBV6-4) TCR (e) on the surface of MR1-5-OP-RU. (f) Ternary complex of an atypical TRAV1-2^{-} TCR (MAV36) and

its footprint (g) on the MR1-5-OP-RU surface. Black-filled circles indicate the centre of mass of the Vα and Vβ domains and the CDR loops are colored as in Figure 1.

Figure 5. Various classes of small molecules chemical scaffolds bound MR1 apart from vitamin B derivatives. Chemical structures (a) and docking of the compounds in the MR1 groove (b) 6-FP, purple; HMB, teal; DCF, pink; 2OH-1-NA, purple; 2,4-DA-6-FP, red; 3-F-SA, orange; and 5-OH-DCF, yellow. Detailed interactions of 3-F-SA (c), 2-OH-1-NA (d) & 5-OH-DCF (e) in the MR1 groove where the agonist 5-OH-DCF is the only one that has a direct contact with the CDR3β of MAIT TCR (f). The orientation of MR1 binding groove in c, d and e are consistent with Figure 3.



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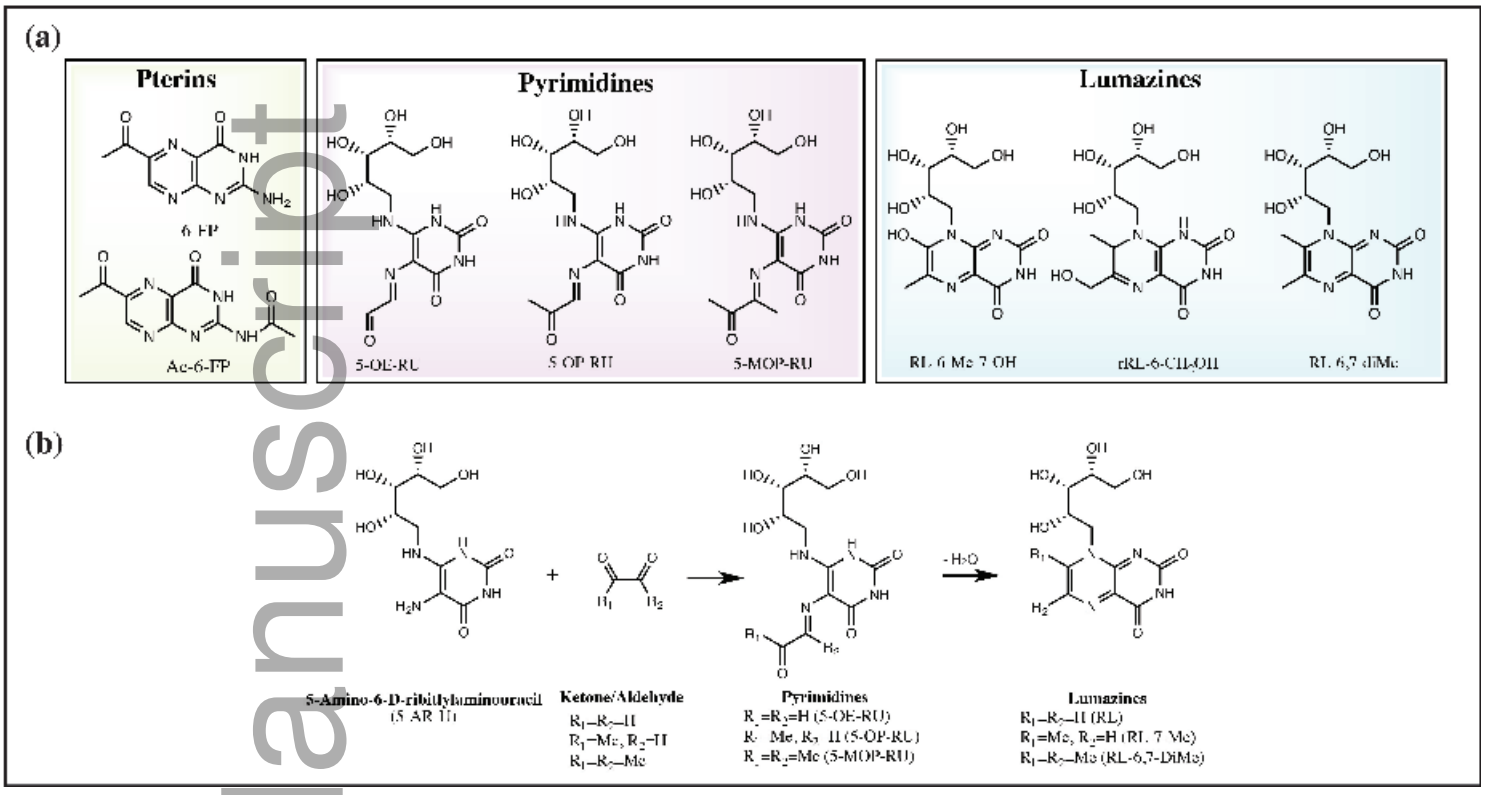

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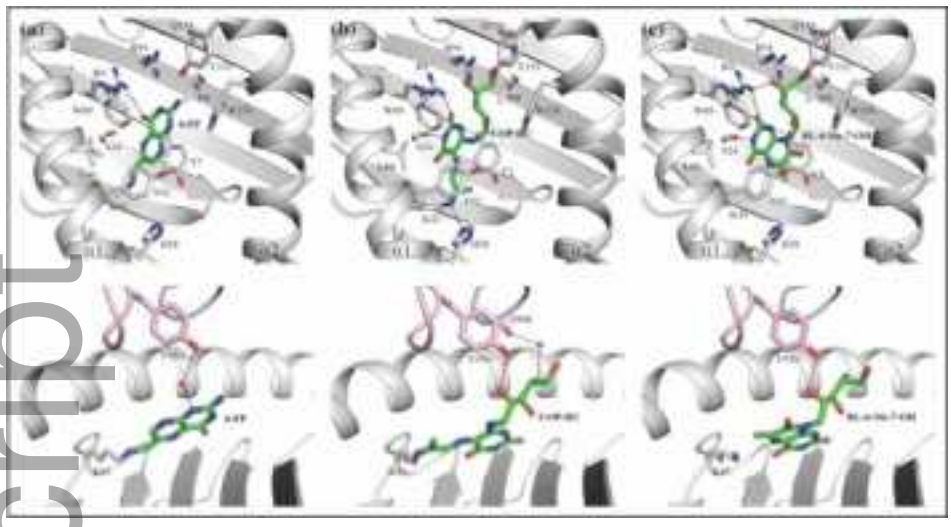

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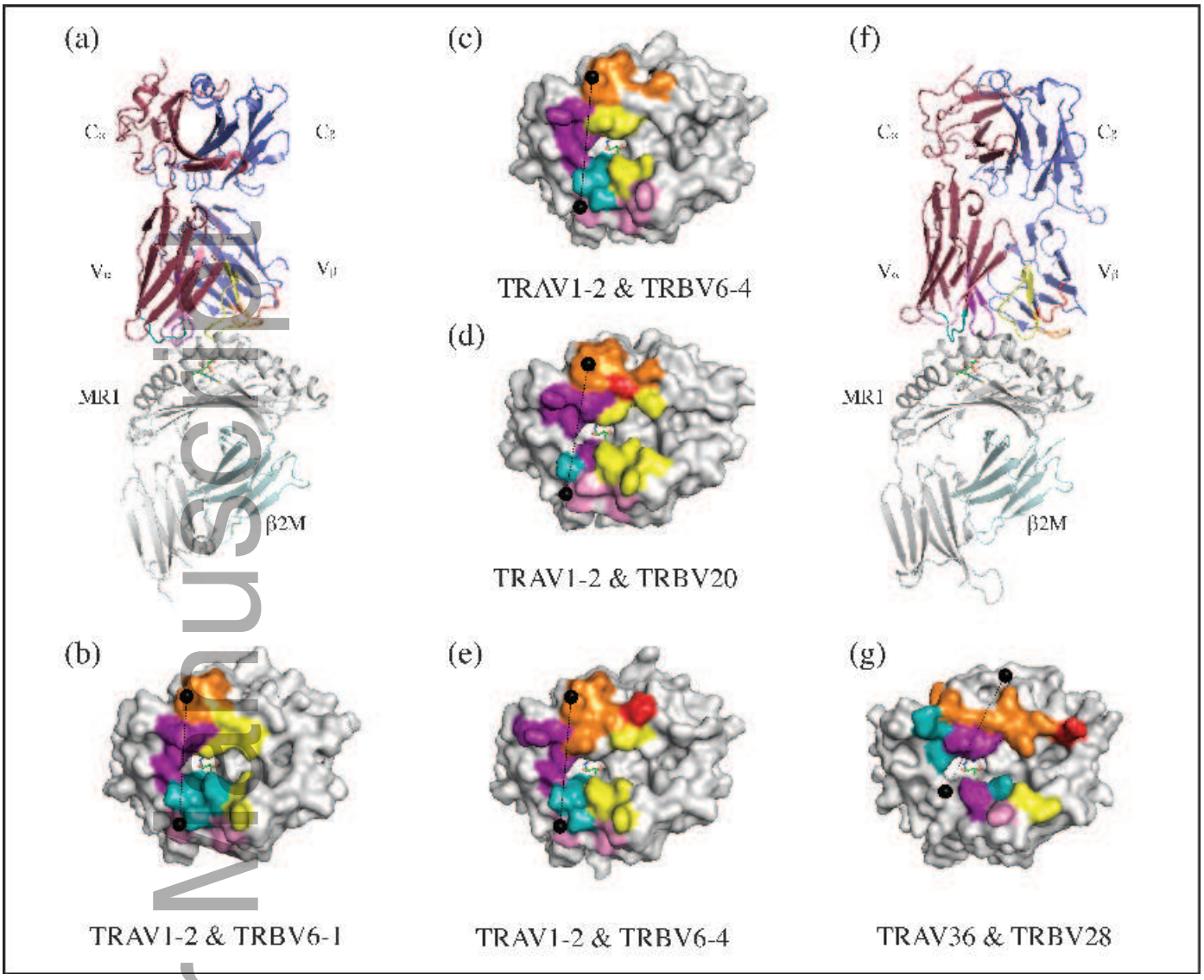

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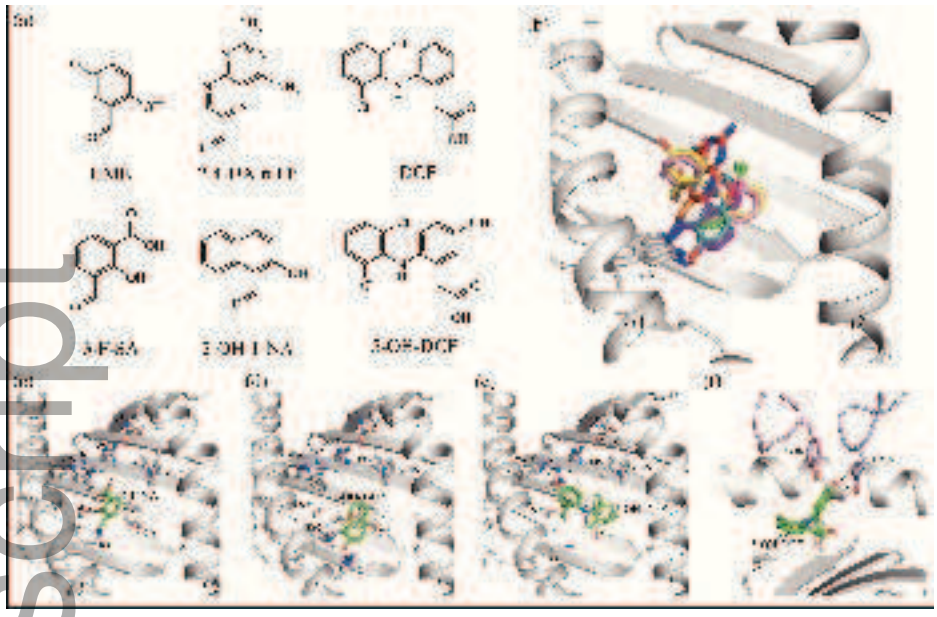

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