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Molecular insights into metabolite antigen recognition by mucosal-associated invariant T cells

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Abstract

Metabolite-based T-cell immunity is emerging as a major player in antimicrobial immunity, autoimmunity, and cancer. Here, small-molecule metabolites were identified to be captured and presented by the major histocompatibility complex class-I-related molecule (MR1) to T cells, namely mucosal-associated invariant T cells (MAIT) and diverse MR1-restricted T cells. Both MR1 and MAIT are evolutionarily conserved in many mammals, suggesting important roles in host immunity. Rational chemical modifications of these naturally occurring metabolites, termed altered metabolite ligands (AMLs), have advanced our understanding of the molecular correlates of MAIT T cell receptor (TCR)-MR1 recognition. This review provides a generalized framework for metabolite recognition and modulation of MAIT cells.

Introduction

Mucosal-associated invariant T (MAIT) cells are a unique subset of innate-like T cells that are evolutionarily conserved across mammalian species [1–3]. Typically, human MAIT cells express a semi-invariant αβ T-cell receptor (TCR), TRAV1–2–TRAJ33/20/12, paired with a limited TCR β-chain repertoire, including TRBV6 or TRBV20 genes [4–10]. During microbial infection, the major histocompatibility complex (MHC) class-I-related protein (MR1) binds to vitamin-B2 derivatives, originating from the biosynthesis pathway of riboflavin, and presents them on the surface of the antigen (Ag)- presenting cells [8,11]. These MR1–Ag complexes are recognized by MAIT TCRs and subsequently stimulate MAIT cells [12–14]. Consequently, MAIT cells are considered to play protective roles in

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Declaration of Competing Interest

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antibacterial immunity [15,16], and have been implicated in auto-immunity and cancer [17–19], making them potentially attractive therapeutic targets [20–22].

During the last few years, several research groups have synthesized and functionally investigated analogs of MR1-binding ligands [23–28] (recent advances in the chemical synthesis of MR1-binding ligands are recently reviewed) [29]. For example, a systematic study was undertaken into how modifications of small-molecule heterocyclic metabolites can impact on T-cell function [23]. Namely, twenty synthetic *altered metabolite ligands* (AMLs) were investigated, in an analogous manner to altered peptide ligands (APLs) and MHC-restricted immunity [23]. Indeed, APLs have proven to be a useful approach to understand the concepts of MHC-restricted T-cell immunity, such as TCR signaling by agonist and antagonist APL [30], and we affirm that the AMLs approach will have crucial implications for future investigations of MAIT cell biology. Here, we review recent advances in the understanding of the MAIT TCR–MR1 axis.

MR1 presents diverse small-molecule metabolites

MR1 is a highly conserved Ag-presenting molecule that is ubiquitously expressed in all cells. However, MR1 upregulation at the cell surface correlates with Ag availability [31–37]. Initially, two classes of vitamin-B-derived compounds were identified as ligands able to be captured and presented by MR1 [11]. The first class included the photodegradation product of folic acid (vitamin B_9), 6-formylpterin (6-FP), and its synthetic derivative, acetyl-6-formylpterin (Ac-6-FP). These metabolites have a bicyclic pterin-based structure with a chemically reactive formyl group (Figure 1). They upregulate MR1 to the cell surface, but do not stimulate the majority of MAIT cells. Both 6-FP and Ac-6-FP inhibit MAIT cell activity, but Ac-6-FP has a 100-fold greater inhibitor potency than 6-FP [6].

The second class of MR1 ligands was derived through a biosynthetic intermediate of riboflavin (vitamin B_2). These metabolites are formed during infection from a plethora of distinct riboflavin-producing microbes, which can potently activate MAIT cells [8,11,38–40]. Vitamin B_2 cannot be synthesized by mammals, thus, the riboflavin-based ligands are defined as a '*molecular signature*' of infection. The microbially derived riboflavin intermediate, 5-amino-6-D-ribitylaminouracil (5-A-RU), can nonenzymatically react with ketones/aldehydes, such as methylglyoxal (pyruvaldehyde) and glyoxal, derived from bacterial metabolism or mammalian glycolysis to produce pyrimidine-based metabolites (Figure 1a–c). These highly potent and unstable ribityl-pyrimidines, such as 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) and 5-(2-oxoethylideneamino)-6-D-ribitylaminouracil (5-OE-RU), can be captured and stabilized by MR1, via formation of a covalent Schiff base with Lys43 of MR1. However, they are also extremely unstable in water (5-OP-RU has half-life time of 88 min of physiological conditions [23]) and undergo cyclization to thermodynamically more stable ribityl-lumazines, such as 7-hydroxy-6-methyl-8-D-ribityllumazine (RL-6-Me-7-OH) and 7-methyl-ribityllumazine (RL-7-Me) [8]. Moreover, Harrif *et al.* identified new ribityl-lumazines as weak MAIT agonists, 6-(2-carboxyethyl)-7-hydroxy-8-ribityllumazine (photolumazine I (PLI)) and 6-(1H-indol-3-yl)-7-hydroxy-8-ribityllumazine (photolumazine III (PLIII)), as well as a new

ribityl-based scaffold of 7,8-didemethyl-8-hydroxy-5-deazariboflavin (FO) (Figure 1c–d) [39•].

Apart from vitamin-B-related MR1 ligands, Keller *et al.* described a panel of drug and drug-like molecules with broad chemical structures that can be displayed by MR1 (Figure 1g) [41]. These included a diverse panel of mono- and bicyclic–chemical structures, such as 2-hydroxy-5-methoxybenzaldehyde (HMB), aspirin analogs (e.g. 3-formyl-salicylic acid (3-F-SA)), a photodegradation product of the chemotherapeutic aminopterin (2,4-diamino-6-formylpterdine (2,4-DA-6-FP)), the sirtinol inhibitor (2-hydroxy-1-naphthaldehyde (2-OH-1-NA)), and the anti-inflammatory drug diclofenac (DCF) and its metabolite 5-hydroxy-diclofenac (5-OH-DCF). Most pharmaceutical metabolites have so far not activated MAIT cells, but DCF metabolites weakly activated some MAIT TCRs. Further, *in silico* screening revealed two uracil-based compounds, 3-[(2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl) formamido] propanoic acid (DB28), and its ester analog, methyl 3-[(2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl) formamido] propanoate (NV18.1), that can bind the MR1 pocket (Figure 1h) [42•]. Most recently, having established a fluorescence-based polarization assay to measure affinity for MR1 ligands in vitro, two diet-derived MR1-binding molecules were identified, vanillin and ethylvanillin (Figure 1f) [43]. Interestingly, a recent report suggested that MR1 recognizes and presents nonmicrobial tumor-cell-derived metabolites, although the ligand identity is yet to be identified [18••].

Geometric features of MR1 binding pocket

X-ray crystal structures of the ternary TCR–MR1–Ags complexes have been published during the last few years, providing some molecular insights into the functions of MR1, MR1-binding ligands, and Ag recognition by the MAIT TCRs [5,6,8,11,23,38,41,44–46]. The MR1–β2m heterodimer complex possesses an Ag-binding cleft (~750$Å^3$) composed of A′ and F′ pockets that are formed between the α1- and α2-helices and sitting atop an anti-parallel β-sheet. All previously recognized ligands were found to bind in the A′ pocket of MR1, where an ‘aromatic cradle’ is formed by aromatic and charged amino acid residues (Figure 2a–c). An unusual feature of the MR1-binding pocket is the ability of the epsilon–amino group of MR1–Lys43 to form a Schiff base with the carbonyl group of a small molecule Ag. MR1–Lys43 is located at the base of MR1-binding cleft and thus the associated ligands are buried deep within the relatively large MR1 cavity. Consequently, all identified MR1-binding ligands to date are not exposed very much on the MR1 surface to T-cell receptors. Intriguingly, some MR1 ligands such as the ribityl-lumazines cannot form a Schiff-base interaction with MR1–Lys43 due to the lack of a reactive carbonyl group and exhibit very low capacity to upregulate MR1 to the surface of Ag-presenting cells [8,11,23]. In the next sections, we will review the capability of the MR1 molecule to capture and display diverse classes of small-molecule metabolites and their recognition by MAIT TCRs.

Mucosal-associated invariant T TCR recognition of ribityl-pyrimidine Ags

The most potent MAIT agonist identified to date is 5-OP-RU, where its α-imminocarbonyl forms a Schiff-base covalent bond with MR1–Lys43 [8]. The amine and carbonyl groups of the 5-OP-RU uracil ring form H-bond with Ser24 and Arg9, respectively, whereby the

entire ring is sandwiched between MR1–Tyr7 and Tyr62 (Figure 2c). The ribityl moiety of 5-OP-RU is stabilized by H-bonding with MR1–Arg9, Arg94, Tyr152, and Gln153. In addition, the ribityl 3′-OH group interacts with the amine group of the uracil ring that together limits the movement of the ribityl moiety within the MR1-binding cleft.

The crystal structure of the typical MAIT TCR–MR1–5-OP-RU complexes shows that the TCR docks centrally atop the A′ pocket of MR1, and the α- and β-chains of the TCR sit over the α2- and α1-helices of MR1, respectively (Figure 2a). The buried surface area (BSA) at the interface between MAIT TCRs and MR1 varied between 1050 and 1200 $Å^2$, with the TCR α- and β-chains contributing almost equally to the BSA of MAIT TCR–MR1 interfaces (Figure 2c). Variations in TRBV utilization within the human MAIT TCR repertoire, such as TRBV6–1, TRBV6–4, and TRBV20, are shown to modestly impact on TCR recognition of MR1. Indeed, most of the previously investigated MAIT TCRs exhibited similar affinities (K_D 2–8 μM) to MR1–5-OP-RU protein as measured by surface plasmon resonance affinity-based binding studies [6]. Moreover, mutagenesis studies to date on MAIT TCRs established that no distinct TCRβ residue was essential for MAIT cell activation [7]. Altogether, this revealed a fine-tuning role of the MAIT TCR β-chain on the MAIT TCR–MR1 recognition axis, whereas the semi-invariant TRAV1–2 α-chain plays a prominent role in MAIT stimulation.

In the crystal structure of MAIT TRAV1–2^+ TCR–MR1–5-OP-RU complexes, 5-OP-RU makes a very small contribution (less than 1%) to the exposed surface area of the MR1–5-OP-RU binary complex for TCR engagement (Figure 2c–e). The ribityl 2′-OH group forms a single H-bond interaction with the evolutionary conserved (TRAJ33/12/20-encoded) Tyr95α from the CDR3α loop of the TRAV1–2 α-chain of MAIT TCRs (Figure 2e). This raised important questions regarding whether the interaction of the Tyr95α of MAIT TCR with the ligand is the sole regulating factor for MAIT activation. If so, why is 5-OP-RU the most potent MAIT agonist, compared with ribityl-lumazines that also carry the same ribityl chain? To answer these questions, we recently investigated how twenty closely related synthetic analogs of 5-OP-RU (or AMLs), impact on the MAIT TCR–MR1 recognition and MAIT stimulation [23,25].

This large panel of AMLs along with intensive biochemical, MR1 upregulation, and MAIT activation assays allowed some general observations to be made on the MAIT TCR–MR1 axis: (1) the precise chemical structure of the Ag ligand and its capacity to form a Schiff-base adduct with MR1 has a profound effect on MAIT-activating potency and propensity to upregulate MR1 cell surface expression. (2) The ribityl moiety of MR1 ligands is required for potent activation of MAIT cells but it is not essential to MR1 upregulation. (3) Removal of the 2′-OH or 3′-OH positions of the ribityl moiety significantly reduced MAIT TCR recognition, whereas removing either the ribityl 4′- or 5′-hydroxyl moieties could be compensated by the others for stimulating MAIT cells. (4) Indeed, no apparent correlation was found between ligand propensity to upregulate MR1 cell surface expression and ligand potency in activating MAIT cells (5).

The structural studies on MAIT TCR–MR1–AML complexes reveal a network of H-bond interactions, featuring an ‘*interaction triad*’ structural motif that is formed between the

Tyr95α from CDR3α loop of the MAIT TCRs, the ribityl moiety, and Tyr152 of MR1 [6,23,38]. This interaction triad is a limiting molecular feature for MAIT stimulation, with its partial or complete disruption resulting in moderate or nonstimulatory MAIT Ags, respectively. Further, site-directed mutagenesis of Tyr95α significantly impacted the MAIT recognition and activation [38]. This suggested that conservation of the 'interaction triad' is a critical molecular feature for MAIT stimulation. In conclusion, MAIT cell activation potency is orchestrated by a network of dynamic compensatory interactions within, and nearby, this MAIT TCR–MR1–Ag 'interaction triad'. New AML analogs have also been described with modifications in the rings of the ligands [24]. It would be interesting to solve the structures of these AMLs to see how they impact the MAIT TCR recognition and the activation triad.

Mucosal-associated invariant T TCR recognition of noncovalent agonists

MAIT-stimulating ligands with moderate potency include the microbial ribityl-lumazines and pharmaceutical DCF metabolites (Figure 1c and g). Structural data for RL-6-Me-7-OH and 5-OH-DCF bound to MR1 revealed that neither formed a Schiff-base adduct with MR1–Lys43 [11,41]. However, Lys43 maintained a similar orientation within the MR1 cleft as when covalently bound to Schiff-based forming ligands (Figure 3). The ribityl chain of 5-OP-RU and of lumazine derivative RL-6-Me-7-OH was in identical positions in the Ag-binding pocket of MR1, with a conserved interaction triad network. Nevertheless, the lumazines were orders- of-magnitude less-potent MAIT agonists than the ribityluracil adducts (Figure 3b). This is likely due to the poor propensity of ribityl-lumazines to upregulate surface MR1 levels, attributable to their inability to form a covalent Schiff base with MR1.

The crystal structure of MAIT A-F7 TCR–MR1–5-OH-DCF shows that the binding mode and bonding contacts of 5-OH-DCF with MR1 differ from the interactions with the riboflavin-based metabolites, where Tyr7 and Trp69 residues had undergone conformational changes to accommodate the ligand (Figure 3c–d). Regarding the TCR contacts, the structural data show a deformation of the interaction triad network at the TCR–MR1 interface, with the 5-OH-DCF forming polar interactions with the CDR3β loop, but not with either TCR CDR3α or MR1–Tyr152, resulting in modest MAIT activation (Figure 3d). This is consistent with variations in the activation potency 5-OH-DCF in diverse Jurkat. MAIT TCR cell lines [41].

MR1 presentation of MAIT non-stimulating metabolites

Through *in silico* screening and in vitro functional assays, diverse sets of MAIT nonstimulating, and even antagonist metabolites have been identified [6,11,41,42]. These include pterin-related compounds, such as 6-FP, Ac-6-FP, and 2,4-DA-6-FP, as well as drug and drug-related metabolites, such as 3-F-SA, 5-F-SA, 2-OH-1-NA, HMB, and the diet-derived natural products, vanillin and ethylvanillin (Figure 1e–g), which can up-regulate MR1 cell surface expression without activating MAIT cells. The crystal structures of their complexes with MAIT TCR–MR1 revealed their ability to covalently bind MR1–Lys43 (Figure 4). These distinct compounds were captured within the A′ cleft of MR1, are wedged

through hydrophobic interactions between Tyr7 and Tyr62 that characteristically form the aromatic cradle. By contrast, the crystal structures of MAIT–MR1–DB28 and NV18.1 show a lack of covalent MR1–Lys43 bond, which accentuates the importance of forming a Schiff-base adduct to refold MR1 that promotes MR1 trafficking to the cell surface (Figures 1h and 4f). This demonstrated that the MR1 pocket was sufficiently adaptable to fit diverse chemical scaffolds of mono- and biheterocyclic ligands.

Within the MAIT TCR–MR1-Ac-6-FP crystal structure, Ac-6-FP forms a H-bond with TCR–Tyr95α, but no interactions were observed between MR1–Tyr152α and either the ligand or TCR–Tyr95α and therefore the TCR–MR1 interaction triad was distorted (Figure 4b). Similarly, all of these MAIT nonstimulating compounds completely disrupted the TCR–MR1 interaction triad, resulting in the absence of MAIT activation by these ligands.

Concluding remarks

The repertoire of the MR1 ligandome is broader than we previously thought, with the MR1-binding cleft displaying enough plasticity to accommodate a wide array of vitamin metabolites and small heterocyclic molecules. In this review, we summarized key factors relating to Ag presentation by MR1 to MAIT cells. Ongoing chemical, structural, and functional studies will provide the rationale for designing MAIT cell activating and inhibiting ligands for potential therapy of immunological and other disorders.

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Data Availability

No data were used for the research described in the article.

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Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest

•• of outstanding interest

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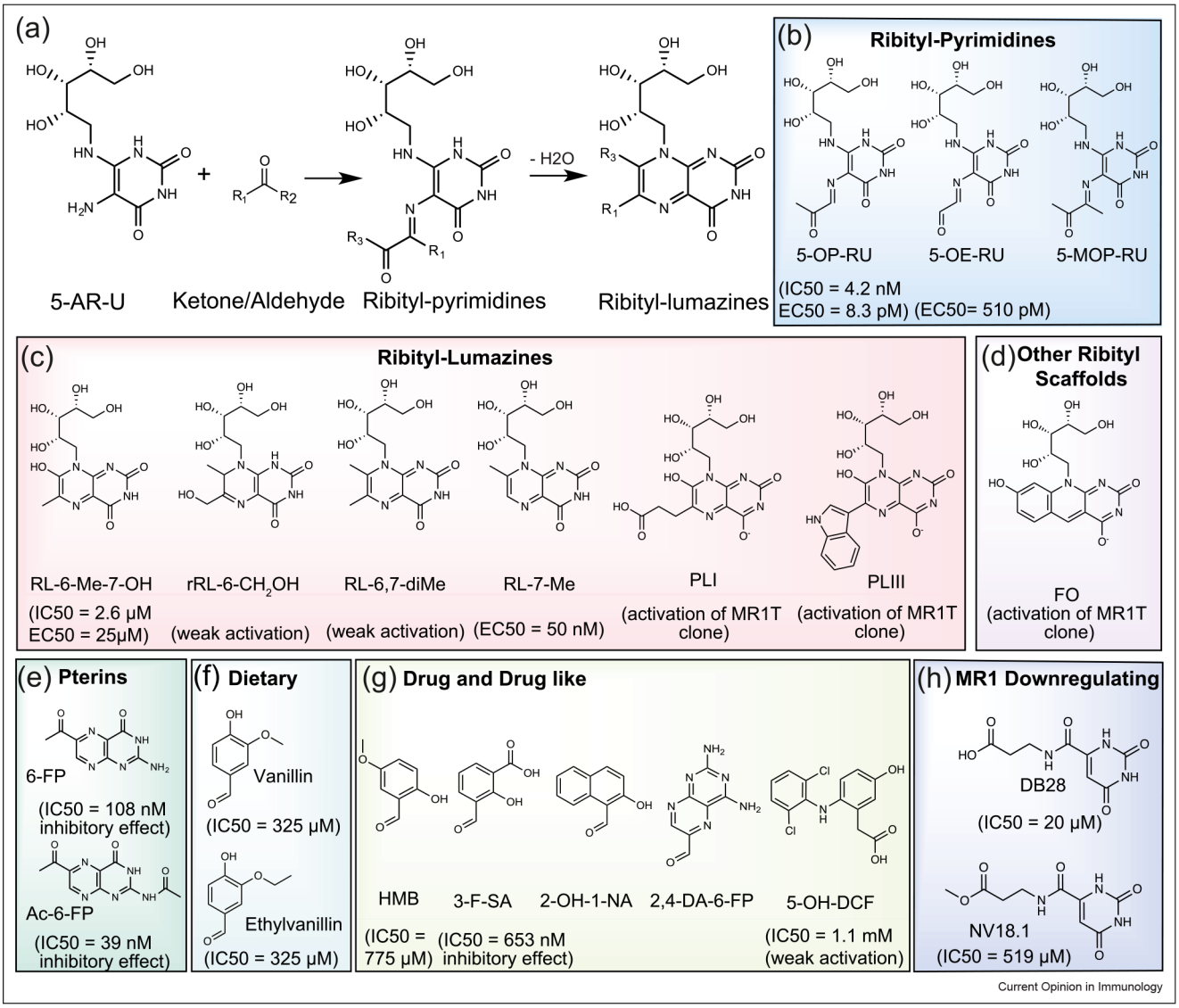

Figure 1.

Broad range of MR1 Ags. **(a)** Condensation reactions of bacterially produced riboflavin intermediate 5-A-RU with reactive ketones/aldehydes producing pyrimidine-based Ags, which subsequently dehydrate to ribityllumazine compounds if not captured by MR1. **(b–h)** Chemical structures of different categories of MR1-binding Ags. **(b–d)** Riboflavin-based Ags. The MR1 ligand affinities (IC50) as measured by the fluorescence-based polarization assay and the activation potency (EC50) of MAIT cells are included. **(b)** Ribityl-pyrimidines: 5-OP-RU, 5-OE-RU, and 5-(1-methyl-2-oxopropylideneamino)-6-D-ribitylaminouracil (5-MOP-RU). **(c)** Ribityl-lumazines: RL-6-Me-7-OH, reduced 6-hydroxymethyl-8-D-ribityllumazine (rRL-6-CH_2OH), 6,7-dimethyl-8-D-ribityllumazine (RL-6,7-diMe), and RL-7-Me, 6-(2-carboxyethyl)-7-hydroxy-8-ribityllumazine (PLI), and 6-(1*H*-indol-3-yl)-7-hydroxy-8-ribityllumazine (PLIII). **(d)** Other ribityl scaffolds: FO. **(e)** Folate-based metabolites, including 6-FP and Ac-6-FP. **(f–h)** Various classes of small-molecule scaffolds aside from vitamin-B derivatives, including **(f)** dietary compounds::

Vallin, and Ethylvanillin; (g) drug-related compounds: HMB, 3-F-SA, 2-OH-1-NA, 2,4-DA-6-FP, and DCF; and **(**h**)** MR1 downregulated compounds: DB28 and NV18.1.

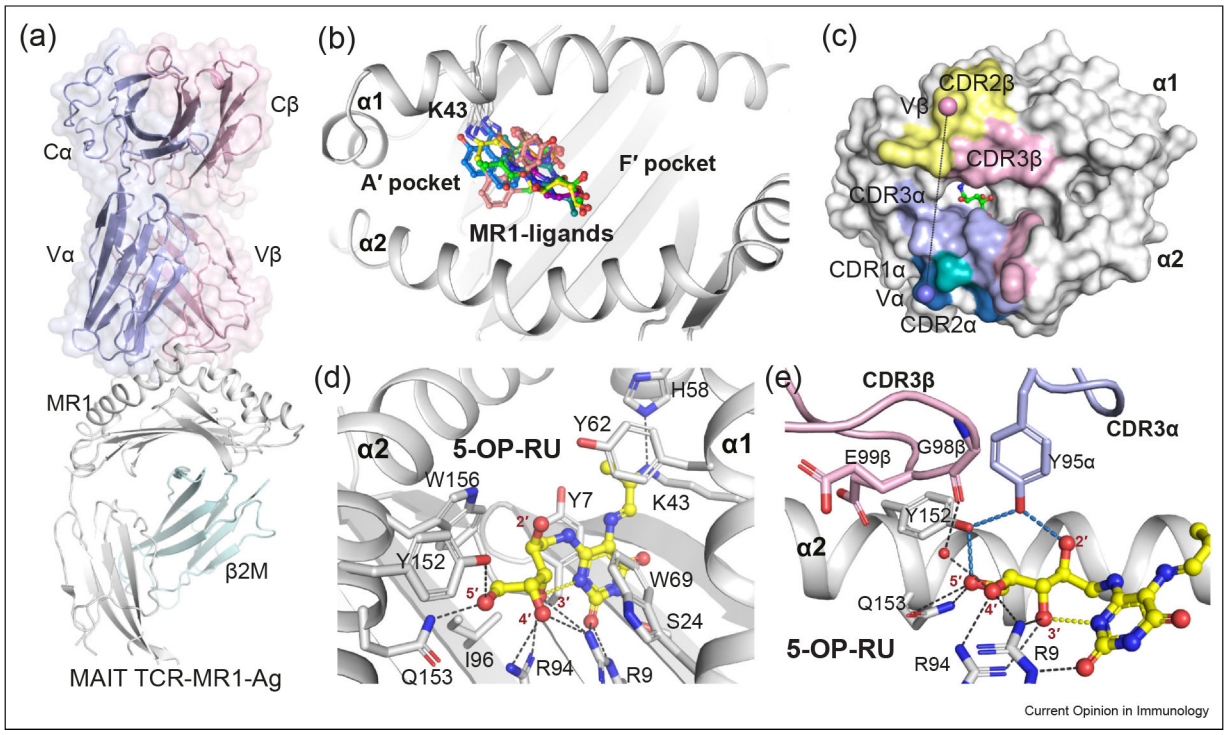

Figure 2.

MAIT TCR recognition of MR1–pyrimidine-based metabolites. **(a)** Cartoon representation of the crystal structure of the ternary complex of the typical MAIT TRAV1–2^{+} A-F7 TCR–MR1–5-OP-RU (PDB: 6PUC). The constant (C) and variable (V) domains of the TCRα and TCRβ chains are shown as light-blue and light-pink surfaces, respectively. The MR1 and β2-microglobulin (β2M) molecules are shown as white and pale-cyan cartoons, respectively. **(b)** Surface representation of the MR1–Ag- binding A′ and F′ pockets that are formed between MR1 α1 and α2-helices. All recognized MR1 ligands (represented as colored sticks), to date, dock in the A′ pocket. **(c)** The footprint of the MAIT A-F7 TCR on the surface of MR1–5-OP-RU. The atomic footprint is colored according to the TCR segment making contact via its complementarity-determining region (CDR) loops colored as follows: CDR1α, teal; CDR2α, sky-blue; CDR3α, light-blue; frameworks of α-chain, dark-green; CDR1β, roseberry; CDR2β, pink; CDR3β, yellow-orange; and frameworks of β-chain, dark-gray. **(d)** Interactions of 5-OP-RU (yellow sticks) within the MR1-binding groove. MR1 α-helices and β-sheets are presented as white cartoon. **(e)** Interactions between the CDR3α and CDR3β loops of the MAIT A-F7 TCR and MR1–5-OP-RU. The relevant intramolecular H-bonds of 5-OP-RU are colored yellow. The MR1 pocket H-bonds are colored black, the TCR-related H-bonds are colored sky-blue. The CDR3α and CDR3β loop are colored as light-blue and light-pink, respectively. Waters are shown as red spheres. The structural illustrations in Figures 2, 3 and 4 were prepared using PyMOL Molecular Graphics System, Version 2.0, Schrodinger.

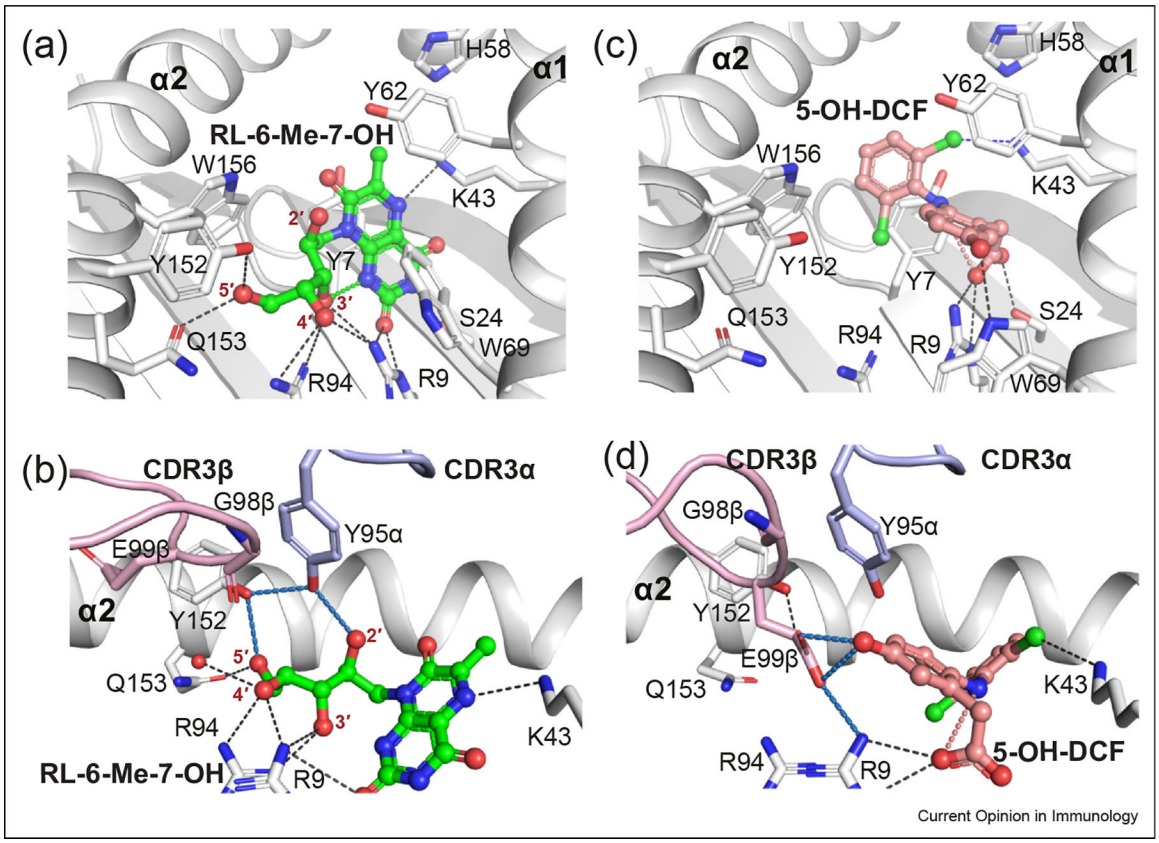

Figure 3.

MR1 presentation of noncovalent MAIT agonists. The RL-6-Me-7-OH **(a–b)** and 5-OH-DCF **(c–d)** metabolites are shown as colored sticks in the MR1-binding pocket (upper panels) and their interactions with A-F7 MAIT TCR (bottom panels) (PDB: 4L4V and 5U72, respectively). RL-6-Me-7-OH and 5-OH-DCF ligands and their intramolecular H-bonds are colored green and salmon, respectively. Halogen bonds are colored in blue. H-bonds, CDR loops are colored as in Figure 2.

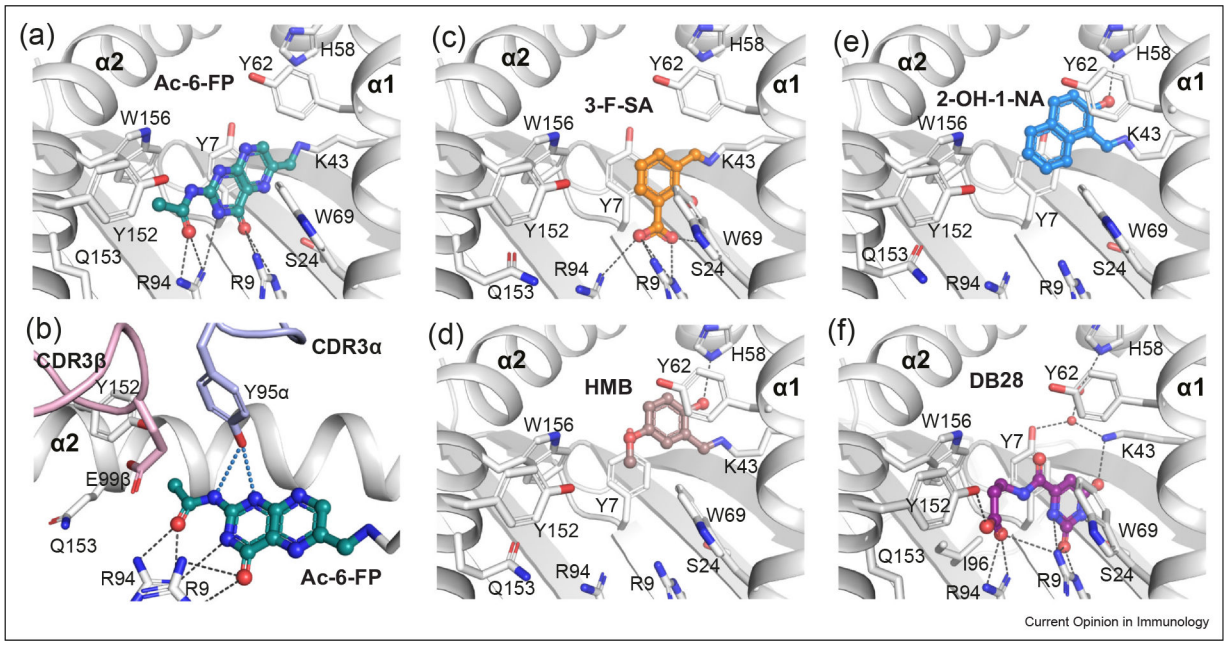

Figure 4.

MR1 presentation of small-molecule MAIT nonstimulating ligands. **(a–b)** Capture of Ac-6-FP ligand (deep-teal sticks) within the MR1-binding cleft **(a)** and its interaction with the CDR loops of MAIT TCR **(b)**. **(c–f)** Docking of diverse chemical identities aside from vitamin-B derivatives within the MR1 groove: **(c)** 3-F-SA (orange), **(d)** HMB (brown), **(e)** 2-OH-1-NA (marine), and **(f)** DB28 (purple). Coloring as in Figure 3 2. Crystal structures of MAIT–MR1 ligands: Ac-6-FP (PDB: 4PJ5), 3-F-SA (PDB: 5U6Q), HMB (PDB: 5U2V), 2-OH-1-NA (PDB: 5U16), DB28 (PDB: 6PVC), and NV18.1 (PDB: 6PVD), used in figure.